THE LINKAGE OF EIGHT SEX-LINKED CHARACTERS IN *DROSOPHILA VIRILIS*

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The present paper deals with eight sex-linked mutant characters that have arisen in my cultures of *Drosophila virilis* Sturtevant.[1] The eight characters are as follows:

Yellow, body color.
Magenta, eye color.
Glazed, eye surface (figure 5).
Forked, bristles on the thorax (figure 2).
Vesiculated, wings.
Rugose, eye surface (figure 4).
Frayed, bands on the abdomen (figures 3 and 6).
Hairy, eye surface (figure 7).

DESCRIPTION OF MUTANT CHARACTERS

The first four of these mutants have been described previously (METZ 1916) and may be passed over briefly. Yellow is distinguished by the yellowish color of the body and wings; magenta by its peculiar eye color; glazed (figure 5) by the varnished or glazed eye surface, and irregular ommatidia; and forked (figure 2) by the forked or stunted bristles on the head and thorax.

The remaining four characters are considered here for the first time, and in connection with their description a word may be said with respect to their origin and initial behavior.

Vesiculated is characterized by the presence of vesicles or blisters in the wings, somewhat like those in the "balloon" wing mutant of *D. ampelophila.* Occasionally the entire wing may be swollen into a single large vesicle filled with liquid, but usually the abnormality is limited to one or two small blisters near the center of the wing. This character was first observed (July, 1916) in several males from a mass culture of normal flies. These males when bred to normal unrelated females gave

[1] For a description of this species see STURTEVANT 1916.

Explanation of Plate 1

The figures are all drawn to the same scale with the aid of a camera lucida, but the individuals were of various sizes due to different cultural conditions.

Figure 1. *Normal,* male. The wing veins and bristles are somewhat exaggerated in this figure.

Figure 2. *Forked,* female.

Figure 3. *Frayed,* male, dorsal view. The wings are cut off to show the abdomen.

Figure 4. *Rugose,* male.

Figure 5. *Glazed,* male.

Figure 6. *Frayed,* male, side view. Wings cut off to show abdomen.

Figure 7. *Hairy.*

Figure 8. *Normal.* Side view for comparison with figure 6. Wings cut off.

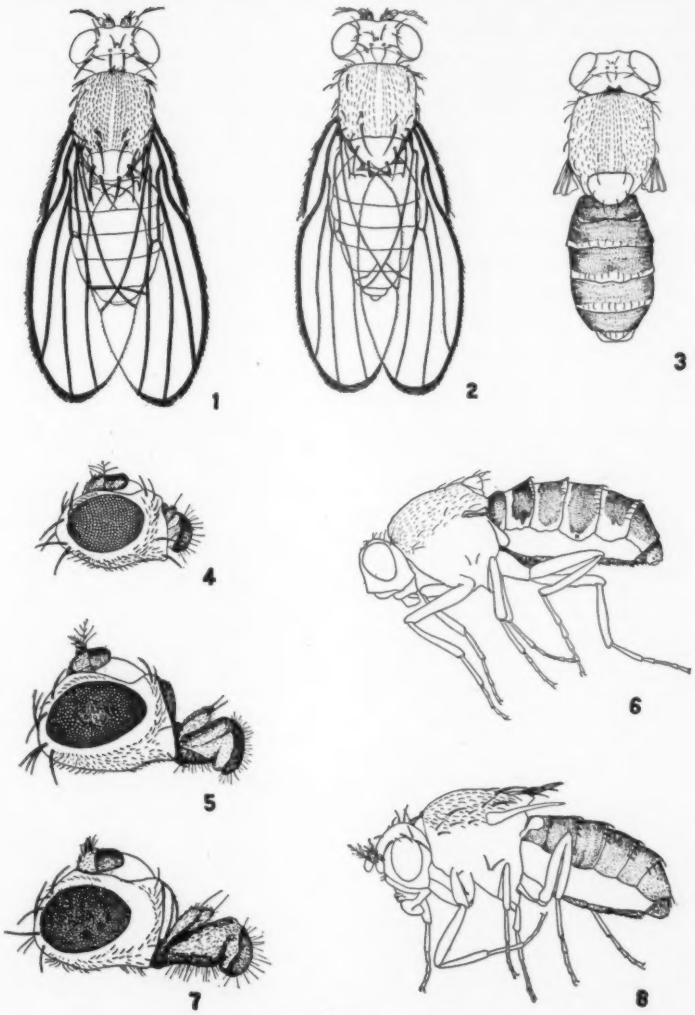


PLATE I

in F_1 normal females and males, and in F_2 224 normal females, 110 normal males and 97 vesiculated males. In F_3 vesiculated females were obtained and a pure stock made up which has subsequently bred true.

The modification called rugose (figure 4) somewhat resembles glazed, but is much less pronounced. The ommatidia of the eye are greatly disarranged but are not fused together in masses as they are in glazed, and there is no smeared or varnished appearance to the surface. Furthermore the eye is full-sized instead of narrow and is decidedly lighter than the normal or the glazed eye. Rugose first appeared in a single male individual (June, 1916) from a mating between a normal female and a confluent bald (non-sex-linked characters) male. This mutant, bred to normal females, gave in F_1 normal males and females, and in F_2 43 normal females, 18 normal males and 11 rugose males. Thus far the behavior was that of a typical sex-linked recessive, but the results in F_3 showed that rugose is only able to exhibit itself in the male sex,—females never show the character, even when homozygous. I now have a pure stock which gives 100 percent rugose males, but only normal (appearing) females.

The next mutant to appear, after rugose, was frayed (figures 3 and 6) —so called because the dorsal bands of pigment on the abdomen are frayed out or irregularly broken at the ends as they extend down the sides of the body. Accompanying this are various other modifications, any one of which might be used in deriving the name of the mutant. Almost the entire fly is affected in some way. The thoracic bristles are reduced to little more than hairs (compare figures 6 and 8); some of the bristles on the head are entirely gone; frequently the aristae, and sometimes the entire antennae are aborted or wanting; the wing veins are frequently broken and disarranged in various ways; and finally the development of the embryo is retarded several days so that in a mixed culture the frayed flies hatch out considerably later than their normal brothers and sisters. This mutant shows a greater variety of modifications than any other I have seen. It originated (September, 1916) in a mass culture containing yellow and normal-colored flies. Among the males in this culture were several, both yellow and brown, showing the frayed character. Two of these frayed males mated to unrelated females gave in F_1 normal males and females, and in F_2 277 normal females, 166 normal males,[2] and 106 frayed males.

The eighth and last character of the series, called hairy eye (figure 7) bears a marked superficial resemblance to rugose, but it lacks the un-

[2] Some of these were yellow.

usually light color of the latter and has a sprinkling of heavy black hairs over the eye surface, giving it a peculiar bushy appearance entirely wanting in rugose. Hairy was first observed (November, 1916) in a single male individual in culture number V745 (a female heterozygous for vesiculated, forked, yellow and glazed, by a normal male). This male was yellow, but showed none of the other three characters. When bred to a normal female he gave in F_1 only normal-eyed flies, and in F_2 normal females and four classes of males as follows: normal 37, hairy 28, yellow 22, yellow hairy 36; or 59 not hairy to 64 hairy, a typical result for a sex-linked recessive. Subsequent matings have indicated that hairy females are completely sterile, and consequently a pure stock cannot be maintained.

LINKAGE

In taking up the question of the linkage relations between these eight characters, it may be well at the outset to state that the general features of linkage in *Drosophila virilis* appear to be entirely comparable with those already well known in *D. ampelophila*. The amount of crossing over, and perhaps certain other details (to be taken up in another paper) differ somewhat in the two species, but there can be no question that in general the process is essentially the same in both. Single crossing over, double crossing over and triple crossing over occur in the sex-linked group of *D. virilis* in much the same manner as in that of *ampelophila;* and when plotted according to their linkage values the factors form a linear series showing the same general features as that of the sex-linked factors in *ampelophila*. The technique employed in this study, and the method of handling the data are, therefore, the same as those already well known from the work on *D. ampelophila* (see MORGAN, STURTEVANT, MULLER and BRIDGES 1915), and need little explanation. Whatever explanation is necessary will be given in its proper connection below.

In actual practice the linkage relations between the mutant characters were determined in the order in which the mutants arose, but in treating the data it is more convenient and more economical of space to take an arbitrary order. A glance at diagram 15 (page 131), showing the factors in their proper linear relations, will indicate the desirability of this. Several of the factors such as yellow and forked, yellow and glazed, frayed and glazed, etc., are so loosely linked as to show almost fifty percent of crossing over, and data involving these combinations alone are of little use in determining linkage values. It is more convenient, therefore, to consider each factor in connection with those adjacent to

it; hence I shall take them up in serial order, beginning with those at the left end of the diagram.

Another arbitrary arrangement that I have adopted in the following pages is the separation of experiments involving only two pairs of characters (giving single crossovers) from those involving three or more pairs (giving multiple crossovers). It is more convenient to make use of the simpler experiments first in approximately determining the serial order of the various factors, and then to take up the more complex crosses to fill in the details. This puts the multiple crossover experiments in a convenient form for future reference, and at the same time makes it possible, by turning to the proper tables, to check up the conclusions derived from single crossover data.

SINGLE CROSSOVER EXPERIMENTS

Since the multiple crossover experiments give more accurate results than the singles, I shall not attempt to treat the latter extensively, but will merely cite a few cases to show the general relations beween the various factors when taken two at a time. In a few cases, where close linkage is involved, this method is fairly accurate, but as a rule it gives only a rough approximation to correct linkage values.

Experiment 1. Yellow and frayed

To determine the linkage between yellow and frayed, females heterozygous for yellow were mated to frayed males, giving daughters part of which were heterozygous for frayed and yellow. Counts were obtained from four females of this kind mated to normal males in pairs. As expected the daughters were all normal, and the sons were of four classes as shown in table 1. Out of 308 males 4 were crossovers, giving a crossover value of approximately 1 percent (shown in the summary of table 1). Expressing this in terms of units, yellow and frayed may be said to be approximately one unit apart, or very closely linked.

TABLE 1

Yellow × *frayed.*

Culture number	N	Y	F_r	$Y\ F_r$
V 810	0	59	36	1
V 811	1	106	68	2
V 814	0	45	25	0
V 847	0	34	21	0
Totals	1	154	150	3

Grand total 308

Summary

Y / F_r	$Y_1\ F_r$
154	3
150	1
304	4

1 percent

Experiment 2. Yellow and vesiculated

Heterozygous females from matings between yellow and vesiculated gave normal females and four classes of males as shown in table 2. In this case there were 35 crossovers (23 percent) out of 151 flies, indicating that yellow and vesiculated are approximately 23 units apart.[3] Knowing the approximate linkage between yellow and frayed, and between yellow and vesiculated, it may be predicted that the serial order of the three factors is either frayed-yellow-vesiculated, or yellow-frayed-vesiculated, not yellow-vesiculated-frayed. Unfortunately the frayed stock was accidentally destroyed at this point in the experiments and the exact position of the factor (whether to the left or the right of yellow) must remain undetermined. (See experiment 14.)

TABLE 2

Yellow × vesiculated.

Culture number	*N*	*V*	*Y*	*YV*
V 470	9	20	21	6
V 484	0	9	6	2
V 503	4	17	24	8
V 507	6	7	12	0
Totals	19	53	63	16

Grand total 151

Summary

$\frac{Y}{V}$	$\frac{Y}{+}$ + $\frac{V}{+}$
53	19
63	16
116	35

23 percent

Experiment 3. Magenta and forked

The characters magenta and forked were early found to be closely linked, and hence may be considered together in relation to those already treated. Table 3 shows the results obtained in F_2 from matings between

TABLE 3

Magenta × forked.

Culture number	*N*	*F*	*M*	*MF*
V 455	2	14	16	0
V 541	1	7	10	1
V 542	1	27	8	0
V 581	2	10	14	1
V 628	2	20	20	0
V 717	0	34	45	2
V 718	1	16	8	0
Totals	9	128	121	4

Grand total 262

Summary

$\frac{M}{F}$	$\frac{M}{+}$ + $\frac{F}{+}$
121	4
128	9
249	13

5 percent

[3] This is only a rough estimate based on very small numbers, and as shown by other experiments is probably five or six units too large.

magenta and forked. Out of 262 flies (males) 13, or approximately 5 percent, are crossovers, indicating that forked and magenta are about 5 units apart.

When tested with yellow, both magenta and forked show such a slight degree of linkage as to indicate that they are probably 50 or more units from that factor. This is shown in tables 4 and 5. When tested with

TABLE 4
Yellow × magenta.

Culture number	*N*	*M*	*Y*	*YM*
V 362	12	20	14	18
V 366	3	7	9	10
V 367	5	12	5	6
V 371	8	31	11	8
V 375	4	12	10	5
V 382	9	19	13	7
V 391	5	8	8	7
V 393	11	19	20	12
V 395	10	18	14	7
V 401	8	10	12	7
V 411	6	10	13	4
V 424	12	13	21	7
V 502	13	15	24	14
Totals	106	194	174	112

Grand total 586

Summary

$\frac{Y}{M}$	$\frac{Y+M}{}$
174	112
194	106
368	218

37 percent

TABLE 5
Yellow × forked.

Culture number	*N*	*F*	*Y*	*YF*
V 462	14	13	17	4
V 473	13	7	17	9
V 476	10	19	24	10
V 492	16	11	19	5
V 493	5	6	11	5
V 498	12	14	29	6
V 501	10	5	6	9
V 509	8	5	17	5
V 510	7	4	8	3
Totals	95	84	148	56

Grand total 383

Summary

$\frac{Y}{F}$	$\frac{Y+F}{}$
148	56
84	95
232	151

40 percent

vesiculated, forked gives nearly the same result as it did with yellow (table 7); but magenta shows a slight amount of linkage (table 6).

From these facts it is possible to conclude provisionally (confirmed by subsequent data) that the position of magenta and forked in the

series is at the right of vesiculated. In other words the order is vesiculated-magenta-forked as shown in diagram 15 (page 131).

TABLE 6

Vesiculated × *magenta.*

Culture number	*N*	*M*	*V*	*MV*
V 445	16	26	19	5
V 479	12	23	19	8
V 487	8	13	11	5
V 505	7	6	7	0
V 607	5	5	2	0
V 659	10	22	25	9
Totals	58	95	83	27

Grand total 263

Summary

$\frac{V}{M}$	$\frac{V}{1}$	$\frac{M}{1}$
83		27
75		58
178		85

32 percent

TABLE 7

(a) *Vesiculated* × *forked.*

Culture number	*N*	*F*	*V*	*VF*
V 466	9	16	9	7
V 471	6	7	8	7
V 478	11	10	15	6
V 481	17	6	13	8
V 620	19	19	29	12
Totals	62	58	74	40

Grand total 234

Summary

$\frac{V}{F}$	$\frac{V}{1}$	$\frac{F}{1}$
58		62
74		40
132		102

(b) *Vesiculated forked* × *normal.*

Culture number	*N*	*F*	*V*	*VF*
V 531	41	17	18	13
V 534	8	2	4	6
V 539	34	17	14	18
V 592	13	8	14	4
V 593	53	24	32	31
V 614	24	9	12	11
V 618	21	4	10	14
V 630	47	12	14	31
Totals	241	93	118	128

Grand total 580

Grand total (a) and (b) 814

Summary

V F	$\frac{V}{F}$
128	118
241	93
369	211

102
211
313

37 percent

Hairy:—See experiment 10

Experiment 4. Forked and rugose

F_2 counts from forked by rugose, obtained in the usual manner, are summarized in table 8. The crossover value in this case is approximately 28 percent. This indicates that rugose must be either 28 units to the

TABLE 8

Forked × rugose.

Culture number	*N*	*F*	*R*	*FR*
V 611	9	13	23	0
V 626	28	36	36	10
V 631	4	13	8	0
V 638	10	11	18	3
V 653	8	11	5	2
V 725	1	14	17	6
Totals	60	98	107	21

Grand total 286

Summary

$\frac{R}{F}$	$\frac{R}{F}$
107	21
98	60
205	81

28 percent

right of forked, or else to the left between vesiculated and magenta. The latter possibility, however, is ruled out by the fact that vesiculated and rugose, when tested together, show almost no linkage. Similarly the multiple crossover results, where rugose is used, all agree in locating it well to the right of forked.

Rugose and glazed

Repeated attempts to cross these two mutants have all failed thus far, so I cannot give the precise location of their factors with respect to one another. But the fact that they give almost identical results in combination with other factors (see experiments 17 and 18) indicates that they are located close together; perhaps they are allelomorphic to one another.

MULTIPLE CROSSOVER EXPERIMENTS

In these experiments it will be possible to secure much more accurate determinations of linkage values than those given above. Both the serial order and the relative "positions" of the factors can thus be ascertained with a fair degree of accuracy. The method of determining the serial order is that already known from the work on *Drosophila ampelophila* (see MORGAN, STURTEVANT, MULLER and BRIDGES 1915). Since the same method is used throughout it need be described only once. This will be done in the first case given below. In the other cases the order will simply be stated, and it will be left to the reader to check up the

statements by referring to the tables. The tables are so arranged as to make this a simple matter.

In order to facilitate comparison of the experiments as a whole I have constructed the graphic summaries shown in diagrams 1-15. Each diagram except the last represents a single experiment. The factors are indicated above the line, and are put in the positions determined by the crossover values of that particular experiment. The factor for yellow, being at one end of the series, is assigned an arbitrary position at zero, and the other factors are located on a line to the right of it. Whenever yellow is absent in an experiment vesiculated is used as a base and assigned the position given by the average of other experiments.

The last diagram is a rough average of the others. It is based largely upon diagrams seven to fifteen, because the preceding ones involve such long distances as to make them unreliable. It should be noted that the position of frayed (*Fr*) may perhaps be at the left of yellow, instead of at the right (see experiment 14), and that the location of rugose may not be exactly the same as that of glazed (see experiment 18).

THREE POINT EXPERIMENTS

Under this heading are grouped the experiments involving three pairs of factors. The first ones to be described are those including yellow; the later ones are those including only factors other than yellow.

Experiment 5. Yellow, magenta and forked

To determine the relations between these three factors yellow magenta males were mated to forked females and the F_1 females bred singly to their brothers or to other males. The sons of these females furnished the desired data.[4] Table 9 gives the results from seven such matings. As may be seen there are eight classes of male offspring. Of these the yellow magenta class and the forked class are non-crossovers; together they give 412, as shown in the summary of classes. The next smaller reciprocal classes are the magenta and the yellow forked. These total 248 as shown in the summary. Next in order of size are the normal and the yellow magenta forked classes, totalling 12 individuals. The remaining two classes, yellow and magenta forked, respectively, include only 5 flies. Assuming that the smallest group represents the double crossovers it is clear that the arrangement of the factors must be in the order yellow-magenta-forked. This being the case the pre-

[4] This method, with few exceptions, has been followed throughout all of the experiments described here. In some instances two or three females were put in the same bottle, but usually these gave about the same results as did single females.

ceding group (normal and yellow magenta forked) represents crossovers between magenta and forked, and the next preceding group represents crossovers between yellow and magenta. By adding the double crossover group to each of the others independently the total number of crossovers between yellow and magenta, and that between magenta and forked are obtained as shown in table 9. These are respectively 37 percent and 3 percent (in round numbers) of the total number of flies. By transposing percentages into units of distance, yellow and magenta are put 37 units apart and forked is put 3 units beyond magenta, as shown in diagram 1, page 131.

TABLE 9

Yellow magenta × forked.

Culture number	*N*	*M*	*F*	*MF*	*Y*	*YM*	*YF*	*YMF*
V 635	0	4	6	0	0	13	2	0
V 755	1	22	33	1	1	38	20	1
V 756	4	27	44	0	0	33	17	1
V 768	1	14	32	1	1	31	21	0
V 779	2	40	43	0	0	56	19	1
V 780	1	23	23	0	1	32	20	0
V 800	0	13	17	0	0	11	6	0
Totals	9	143	198	2	3	214	105	3

Grand total 677

Summary of classes

$\frac{YM}{F}$	$\frac{M}{YF}$	$\frac{YMF}{\ }$	$\frac{MF}{Y}$
214	143	3	2
198	105	9	3
412	248	12	5

Summary of crossovers

Y–M	*M–F*
248	12
5	5
253	17
37%	3%

Experiment 6. Yellow, vesiculated and forked

In this experiment vesiculated females were mated to yellow forked males and the F_2 sons recorded as shown in table 10. Only 187 flies were obtained, and hence the results are subject to considerable error, but they are sufficient to show the relative positions of the factors concerned, and, as may be seen, they agree approximately with those from other experiments.

The crossover values in this case are 22 percent between yellow and vesiculated, and 32 percent between vesiculated and forked, or a total

of 54 units between yellow and forked. As may be noted the 54 units here represent a wide deviation from the 40 units between yellow and forked in the preceding experiment. This is due, as will appear later, to the presence of at least one long "distance" in each case, giving opportunity for many undetectable double crossovers to occur. Especially is this true of the experiment involving yellow, magenta and forked, for yellow and magenta are in reality 50 or more units apart. Double crossing over here greatly reduces the apparent crossover value.

TABLE 10

Yellow forked × vesiculated.

Culture number	*N*	*F*	*V*	*FV*	*Y*	*YF*	*YV*	*YVF*	Total
V 642	1	2	17	1	6	12	3	1	43
V 644	0	3	10	6	8	5	2	2	36
V 658	3	3	8	4	2	5	2	1	28
V 680	4	1	17	5	4	4	1	0	36
V 683	0	1	4	1	4	5	0	2	17
V 697	2	4	9	1	2	6	3	0	27
Totals	10	14	65	18	26	37	11	6	187

Summary of classes

$\frac{Y\ F}{V}$	$\frac{Y\ V}{F}$	$\frac{Y}{V\ F}$	$\frac{Y\ V\ F}{}$
29	5	7	2
15	5	14	2
13	5	6	4
21	2	9	4
9	1	5	2
15	7	3	2
102	25	44	16

Summary of crossovers

Y-V	*V-F*
25	44
16	16
41	60
22%	32%

Experiment 7. Yellow, vesiculated and magenta

This experiment differs little from the preceding, since magenta and forked are only a few units apart. Table 11 shows the results from the few counts obtained. Yellow and vesiculated in this case give a crossover value of 17 percent, as compared with 22 percent in the former experiment. Vesiculated and magenta give a value of about 30 percent, placing magenta 30 units beyond vesiculated. This is slightly less than the distance between vesiculated and forked, and harmonizes with the results given in the yellow magenta forked cross (experiment 5).

TABLE II

Yellow vesiculated magenta.

(a) Yellow × vesiculated magenta

Culture number	*N*	*M*	*V*	*VM*	*Y*	*YM*	*YV*	*YVM*	Total
V 512	3	1	5	6	10	2	4	1	32
V 518	1	1	7	9	13	3	3	0	37
Totals	4	2	12	15	23	5	7	1	69

Summary of classes

$\frac{Y}{VM}$	$\frac{Y}{Y}\frac{}{VM}$	$\frac{Y}{V}\frac{M}{}$	$\frac{}{Y}\frac{}{V}\frac{M}{}$
23	4	5	2
15	1	12	7
38	5	17	9

(b) Yellow vesiculated × magenta

Culture number	*N*	*M*	*V*	*VM*	*Y*	*YM*	*YV*	*YVM*	Total
V 594	7	18	3	1	2	4	20	6	61
V 622	3	8	1	0	1	2	5	2	22
Totals	10	26	4	1	3	6	25	8	83

$\frac{YV}{M}$	$\frac{V}{Y}\frac{}{M}$	$\frac{YV}{}\frac{M}{}$	$\frac{}{Y}\frac{V}{}\frac{M}{}$
25	4	8	1
26	6	10	3
51	10	18	4

(c) Vesiculated × yellow magenta

Culture number	*N*	*M*	*V*	*VM*	*Y*	*YM*	*YV*	*YVM*	Total
V 619	0	3	11	7	2	11	1	0	35

Grand total 187

$\frac{V}{Y}\frac{}{M}$	$\frac{Y}{}\frac{V}{M}$	$\frac{V}{Y}\frac{M}{}$	$\frac{}{Y}\frac{}{V}\frac{M}{}$
11	1	7	0
11	3	2	0
22	4	9	0
111	19	44	13

Summary of crossovers

Y–V	*V–M*
19	44
13	13
32	57
17%	30%

Experiment 8. Yellow, forked and glazed

This experiment is of value mainly in showing the long "distances" between the respective factors used. The number of flies here (451) is sufficient to give fairly accurate results, so far as accurate results may be obtained from such a mating, but as may be seen (diagram 4) the

crossover values—39 between yellow and forked, and 25 between forked and glazed—are so large as necessarily to be approximations only.

TABLE 12

Yellow glazed × *Forked*

Culture number	*N*	*F*	*G*	*GF*	*Y*	*YF*	*YG*	*YGF*	Total
V 488	7	6	7	2	7	1	14	0	44
V 499	4	3	3	2	5	4	11	4	36
V 524	3	15	13	9	8	9	12	5	74
V 529	1	6	6	0	2	4	4	0	23
V 536	1	4	5	0	3	1	6	0	20
V 540	3	2	3	1	8	4	8	0	29
V 543	3	5	8	0	3	2	8	0	29
V 564	5	7	3	1	6	4	7	0	33
V 571	0	7	6	0	2	5	10	3	33
V 583	0	3	1	0	3	4	7	0	18
V 649	6	26	22	0	6	15	37	0	112
Totals	33	84	77	15	53	53	124	12	451

Summary of classes

Y G / *F*	*G* / *Y F*	*Y* / *F G*	*Y F G*
20	8	9	7
14	7	7	8
27	22	17	8
10	10	2	1
10	6	3	1
10	7	9	3
13	10	3	3
14	7	7	5
17	11	2	3
10	6	3	0
63	37	6	6
208	131	68	45

Summary of crossovers

Y–F	*F–G*
131	68
45	45
176	113
39%	25%

Experiment 9. Yellow, vesiculated and glazed

Eight females from a cross of vesiculated female by yellow glazed male gave 579 male offspring as summarized in table 13. The crossover percentage between yellow and vesiculated is in this case 16, as compared with 17 and 22 in the preceding experiments. The percentage between vesiculated and glazed is 40,—i.e., practically no linkage. In reality the "distance" between these latter two is much greater, as shown by other experiments, but is concealed here by undetected double crossing over.

TABLE 13

Yellow glazed × vesiculated.

Culture number	*N*	*V*	*G*	*GV*	*Y*	*YV*	*YG*	*YGV*	Total
V 666	3	18	4	9	13	7	25	1	80
V 667	1	17	3	7	17	4	17	3	69
V 668	0	6	1	3	1	2	2	1	16
V 669	7	15	4	8	17	3	9	0	63
V 670	2	19	2	16	19	3	22	0	83
V 671	7	33	5	19	17	7	34	1	123
V 672	3	26	5	10	20	3	27	2	96
V 701	5	15	1	4	13	2	9	0	49
Totals	28	149	25	76	117	31	145	8	579

Summary of classes

$\frac{Y \quad G}{V}$	$\frac{\quad G}{Y \mid V}$	$\frac{Y}{V \mid G}$	$\frac{\quad\mid\quad\mid}{Y \mid V \mid G}$
43	11	22	4
34	7	24	4
8	3	4	1
24	7	25	7
41	5	35	2
67	12	36	8
53	8	30	5
24	3	17	5
294	56	193	36

Summary of crossovers

Y–V	*V–G*
56	193
36	36
92	229
16%	40%

Experiment 10. Yellow, hairy and forked

Hairy has been tested in only two combinations. The first, with yellow and magenta, indicated that hairy was closely linked to magenta. Only 62 flies were secured in this mating, so the data are not given in detail. In the second case yellow hairy males were mated to forked females. This time 162 F_2 males were secured. They fall into only six classes, due to the entire absence of double crossover individuals (table 14). The missing classes are hairy forked and yellow, indicating that hairy lies to the left of forked and is very closely linked to it. Only five crossovers between hairy and forked are represented among the 162 flies. This would give hairy a position about 3 units from forked, or about half way between forked and magenta; and until further data are obtained it may provisionally be located here.

Experiment 11. Vesiculated, magenta and rugose

In this experiment, as in number 9, long distances are involved. Table 15 is a summary of the F_2 males from a cross of magenta female by vesiculated rugose male. The crossover percentage between vesiculated

TABLE 14

Yellow hairy × *forked.*

Culture number	*N*	*H*	*F*	*HF*	*Y*	*YH*	*YF*	*YHF*
V 1041	0	14	18	0	0	19	13	2
V 1042	2	17	20	0	0	35	21	1
Totals	2	31	38	0	0	54	34	3

Summary of classes

YH / *F*	*H* / *Y* — *F*	*YH* — *F*	*H* — *Y* — *F*
54	31	3	0
38	34	2	0
92	65	5	0

Summary of crossovers

Y–H	*H–F*
65	5
40%	3%

TABLE 15

Magenta × *vesiculated rugose.*

Culture number	*N*	*M*	*R*	*RM*	*V*	*VM*	*VR*	*VRM*	Total
V 659	5	26	10	4	3	7	25	0	80
V 749	2	16	4	1	5	7	11	0	46
V 753	2	15	6	6	2	6	6	2	45
V 758	2	26	7	7	8	6	13	4	73
V 764	2	16	9	4	5	10	22	1	69
V 773	1	18	9	2	7	10	17	1	65
V 822	3	23	8	5	5	12	27	2	85
Totals	17	140	53	29	35	58	121	10	463

Summary of classes

M / *V* — *R*	*V* — *M* / *R*	*M* — *R* / *V*	*V* — *M* — *R*
51	17	7	5
27	11	6	2
21	12	8	4
39	13	15	6
38	19	9	3
35	19	9	2
50	20	10	5
261	111	64	27

Summary of crossovers

V–M	*M–R*
111	64
27	27
138	91
29%	19%

and magenta is 29, as compared with 30 in experiment 7. That between magenta and rugose is 19. Both of these values are unduly small, even when allowance is made for double crossovers. Apparently crossing over was decreased in the whole experiment by some environmental or other cause undetected at the time.

Experiment 12. Vesiculated, magenta and forked

This experiment differs from the preceding in that forked takes the place of rugose. A forked female mated to vesiculated magenta males gave normal daughters whose male offspring are shown in table 16. The crossover values are 37 between vesiculated and magenta, as compared with 29 and 30 in preceding cases, and 5 between magenta and forked as compared with 3 and 5 in preceding cases. A much closer approxima-

TABLE 16

Forked × vesiculated magenta.

Culture number	*N*	*M*	*F*	*MF*	*V*	*VM*	*VF*	*VMF*	Total
V 646	1	9	5	1	0	13	3	0	32
V 657	2	12	20	1	0	16	5	0	56
V 676	1	4	3	0	0	14	4	0	26
V 679	0	7	5	0	0	10	6	2	30
V 715	0	8	1	0	0	8	5	0	22
V 716	0	12	19	0	0	24	15	3	73
V 722	1	14	17	2	1	34	15	1	85
V 723	2	22	20	0	0	28	9	0	81
V 724	1	7	11	0	0	15	10	1	45
V 728	0	5	12	0	0	9	3	0	29
V 732	2	6	7	1	0	6	3	0	25
Totals	10	106	120	5	1	177	78	7	504

Summary of classes

F / *VM*	*V* + *F* / *M*	*VM* + *F*	*V* + / *M* + *F*
18	12	1	1
36	17	2	1
17	8	1	0
13	13	2	0
9	13	0	0
43	27	3	0
51	29	2	3
48	31	2	0
26	17	2	0
21	8	0	0
13	9	2	1
297	184	17	6

Summary of crossovers

V–M	*M–F*
184	17
6	6
190	23
37%	5%

tion to correct linkage values is evident in this experiment because the relatively short "distance" between magenta and forked makes it possible to detect most of the crossovers.

Experiment 13. Vesiculated, forked and rugose

In this experiment a vesiculated rugose female was mated to forked males. The F_2 male offspring are summarized in table 17. Although the count is small (163 flies) the relative values harmonize fairly well with those in other experiments. The crossover percentage between vesiculated and forked is about 38, in comparison to 42 (37 plus 5) in the preceding experiment and 32 in experiment 6. The percentage between forked and rugose is 27, the most accurate determination of this distance thus far made.

TABLE 17

Vesiculated forked × rugose.

Culture number	*N*	*F*	*R*	*FR*	*V*	*VF*	*VR*	*VFR*	Total
V 726	8	9	11	3	4	13	13	3	64
V 727	5	4	15	0	2	10	5	7	48
V 731	5	6	19	2	2	5	16	6	61
Totals	18	19	45	5	8	28	34	16	173

Summary of classes

$\frac{VF}{R}$	$\frac{F}{V \quad R}$	$\frac{VF \quad R}{\quad}$	$\frac{F \quad R}{V \quad \quad}$
24	22	11	7
25	9	12	2
24	22	11	4
73	53	34	13

Summary of crossovers

V–F	*F–R*
53	34
13	13
66	47
38%	27%

Experiment 14. Frayed, vesiculated and magenta

In the cross between yellow and frayed described above (experiment 1) it was shown that frayed is very closely linked to yellow. The present experiment summarizes the only other linkage data obtained from frayed before the stock was lost. It is of interest mainly in corroborating the other experiment as to the position of frayed.

Frayed males mated to vesiculated magenta females gave F_2 males as shown in table 18. According to these data the factor for frayed

lies 18 units to the left of vesiculated. This is within one or two units of the position of yellow as given in the six experiments involving vesiculated and yellow. But since the value fluctuates more than two units in different experiments the figures do not determine whether frayed lies at the left or the right of yellow.

TABLE 18

Vesiculated magenta × frayed.

Culture number	*N*	*M*	*V*	*VM*	*Fr*	*FrM*	*FrV*	*FrVM*	Total
V 951	6	4	11	17	18	9	2	2	
V 953	6	3	13	29	34	21	2	4	
V 954	4	3	7	11	9	3	1	1	
V 955	7	3	5	20	20	14	6	2	
Totals	23	13	36	77	81	47	11	9	296

Summary of classes

VM / *Fr*	*Fr* : *VM*	*V* : *Fr* *M*	*Fr* : *V* *M*
35	8	20	6
63	10	34	5
20	5	10	4
40	9	19	8
158	32	83	23

Summary of crossovers

Fr-V	*V-M*
32	83
23	23
55	106
18%	35%

FOUR POINT EXPERIMENTS

Under this heading are included the experiments involving four pairs of factors. These give sixteen classes of males in F_2—furnishing triple crossovers in addition to the doubles and singles of three point crosses. They are the most significant experiments of all, since they make it possible to break up the long "distances" into their shorter components and analyse them more accurately than in the two or three point crosses.

It will not be necessary, however, to consider each mating in detail. The methods are essentially the same as those used in three point crosses. In each case the heading of the table indicates how the cross was made, and the table includes only the F_2 males. The crossover value for any two factors is computed from the sum of single, double and triple crossovers involving the region between them.

Experiment 16. Yellow, vesiculated, magenta and forked

Alternate factors were introduced from the same side in this experiment by mating yellow magenta females to vesiculated forked males. The sons were all yellow magenta and the daughters normal. From the latter, sixteen classes of sons were obtained as summarized in table 19. By adding complementary classes eight groups are obtained as shown in the lower part of the table. The first of these is the non-crossover group; then follow the three single crossover groups, the three

TABLE 19

Yellow magenta × vesiculated forked.

Culture number	*N*	*M*	*F*	*V*	*MF*	*MV*	*VF*	*VM F*	*Y*	*YM*	*YF*	*YV*	*YM F*	*YV M*	*YV F*	*YV MF*	Totals
V 754	1	5	3	1	0	7	30	0	1	22	16	0	0	2	8	0	96
V 762	0	6	4	2	0	22	31	1	2	34	21	2	1	2	3	0	131
V 781	0	8	6	2	0	18	23	0	2	36	16	0	0	5	11	0	127
V 785	0	1	2	0	0	13	19	1	0	17	12	0	2	5	3	0	75
V 786	0	4	0	0	0	17	21	0	0	18	5	0	0	3	8	0	76
V 787	1	5	0	0	0	14	31	0	0	32	12	1	1	1	1	0	99
V 798	1	0	0	0	0	4	9	0	0	5	10	0	2	1	1	0	33
V 825	0	5	2	0	1	3	15	0	1	14	9	0	0	2	5	0	57
V 833	0	1	2	0	0	2	5	0	1	14	7	0	0	4	0	0	36
V 901	0	2	1	1	0	5	12	0	0	14	2	0	0	2	2	0	41
V 902	0	2	0	0	0	3	11	0	0	11	8	0	1	0	1	0	37
V 903	0	3	0	0	0	6	8	1	0	31	12	0	2	0	3	0	66
V 905	0	2	1	1	0	6	8	0	0	8	1	1	0	0	3	0	31
V 906	0	4	3	1	0	10	14	0	0	22	6	0	1	2	3	0	66
V 907	1	9	0	1	1	3	20	1	0	20	9	0	0	4	2	0	71
V 919	0	3	1	0	0	9	12	0	1	9	8	1	0	0	0	0	44
Totals	4	60	25	9	2	142	269	4	8	307	152	5	10	33	54	0	1086

Summary of classes

	$\frac{Y\ M}{V\ F}$	$\frac{M}{Y\ VF}$	$\frac{V\ M}{Y\ F}$	$\frac{Y\ M\ F}{V}$	$\frac{Y\ V\ M}{F}$	$\frac{Y\ V}{M\ F}$	$\frac{Y}{V\ M\ F}$	$\frac{Y\ V\ M\ F}{}$
	307	60	142	10	33	5	8	0
	269	54	152	9	25	2	4	4
Totals	576	114	294	19	58	7	12	4

Summary of crossovers

	Y-V	*V-M*	*M-F*
	114	294	19
	58	58	7
	7	12	12
	4	4	4
Totals	183	368	42
	17%	34%	4%

double crossover groups and finally the triple crossover group. As may be seen no other arrangement of the factors than that given will account for the graduated size of the classes.

By determining the percentage of crossing over as in preceding cases the "distances" are given as follows: yellow to vesiculated 17 units; vesiculated to magenta 34 units; magenta to forked 4 units. How well these harmonize with other experiments may be seen by comparing diagram 11 with diagrams 2, 3 and 8.

Experiment 17. Yellow, vesiculated, forked and glazed

Table 20 and diagram 12 summarize the main features of this experiment. Vesiculated forked females were mated to yellow glazed males and the F_2 males recorded. These are in fifteen classes,—one expected triple crossover class (yellow vesiculated) being absent. Summarized in the same manner as before the crossover data are as follows: Non-crossovers 109; single crossovers between yellow and vesiculated 29;

TABLE 20

Yellow glazed × vesiculated forked.

Culture number	*N*	*V*	*F*	*G*	*VF*	*VG*	*FG*	*VFG*	*Y*	*YV*	*YF*	*YG*	*YVF*	*YVG*	*YFG*	*YFVG*	Total
V 719	1	3	2	7	15	10	0	5	12	0	19	14	4	1	5	1	99
V 721	2	6	2	2	18	8	0	4	6	0	5	18	4	0	4	2	81
V 721-2	0	5	3	3	17	8	1	2	7	0	9	9	6	2	1	0	73
V 745	1	4	0	2	7	5	0	0	7	0	6	11	1	0	1	0	45
Totals	4	18	7	14	57	31	1	11	32	0	39	52	15	3	11	3	298

Summary of classes

Y G / *VF*	*G* / *Y VF*	*Y F* / *V G*	*V* / *VF G*	*Y V G* / *F*	*Y F G* / *V*	*Y VF G*	*Y V* / *F G*
52	14	39	32	3	11	3	0
57	15	31	11	7	18	4	1
109	29	70	43	10	29	7	1

Summary of crossovers

	Y-V	*V-F*	*F-G*
	29	70	43
	10	10	29
	7	29	7
	1	1	1
	47	110	80
Totals	16%	37%	27%

singles between vesiculated and forked 70; singles between forked and glazed 43; doubles involving the regions yellow-vesiculated and vesiculated-forked 10; those involving the regions vesiculated-forked and forked-glazed 29; those involving the regions yellow-vesiculated and forked-glazed 7; triple crossovers 1. Adding the crossovers for each region independently and determining the percentages, the results are: 16 percent between yellow and vesiculated, 37 percent between vesiculated and forked, and 27 percent between forked and glazed—a very close approximation to corresponding regions in the preceding experiment.

Experiment 18. Yellow, vesiculated, forked and rugose

Except for the substitution of rugose in place of glazed this is a repetition of the preceding cross; and as seen in the summary (table 21) and

TABLE 21

Vesiculated forked × yellow rugose.

Culture number	*N*	*F*	*R*	*V*	*FR*	*VF*	*VR*	*VFR*	*Y*	*YF*	*YR*	*YV*	*YFR*	*YVF*	*YVR*	*YVFR*	Totals
V 783	2	5	1	7	0	16	9	4	14	9	24	4	5	1	0	1	102
V 784	1	0	5	5	1	12	13	5	6	15	13	0	3	0	1	1	81
V 803	4	5	5	5	0	22	23	7	13	15	28	3	1	8	3	0	142
V 804	0	6	2	6	1	14	4	2	10	10	9	0	1	5	1	1	72
V 819	0	2	5	5	1	16	15	8	11	16	25	1	4	6	3	1	119
V 820	6	1	4	1	0	19	11	0	9	9	22	2	4	1	1	1	91
V 826	0	1	3	6	0	11	3	4	7	11	9	3	0	2	1	1	62
V 831	1	0	3	1	0	3	3	2	1	1	12	0	0	3	0	0	30
Totals	14	20	28	36	3	113	81	32	71	86	142	13	18	26	10	6	699

Summary of classes

VF	*Y* | *VF*	*V* | *R*	*VF* | *R*	*F*	*V*	*Y* | *VF* | *R*	*V* | *V*
Y *R*	*R*	*Y* | *F*	*Y*	*V* | *F* | *R*	*Y* | *F* | *R*		*F* | *R*
113	26	81	32	20	36	6	13
142	28	86	71	10	18	14	3
255	54	167	103	30	54	20	16

Summary of crossovers

Y-V	*V-F*	*F-R*
54	167	103
30	30	54
20	54	20
16	16	16
120	267	193
17%	38%	27%

in diagram 13, the respective percentages are almost exactly the same. These two experiments leave little doubt that glazed and rugose are very closely linked.

Experiment 19. Yellow, vesiculated, magenta and rugose

In this experiment magenta was substituted for forked, and the factors were introduced in different combinations from those given in the last case. Yellow magenta females were mated to vesiculated rugose males and the grandsons recorded in the usual manner (table 22). The crossover values are: 20 percent between yellow and vesiculated, 29 percent between vesiculated and magenta, and 29 percent between magenta and rugose. Each of these deviates two or three percent from what

TABLE 22

Yellow magenta × vesiculated rugose.

Culture number	*N*	*M*	*R*	*V*	*MR*	*MV*	*RV*	*RMV*	*Y*	*MY*	*RY*	*VY*	*RMY*	*VMY*	*RVY*	*VMRY*	Total
V 909	0	2	1	6	5	7	15	2	3	20	11	1	7	1	5	3	89
V 910	0	3	1	3	0	0	7	1	2	10	4	1	4	0	1	0	37
V 911	0	5	4	9	5	12	17	3	5	23	14	3	5	2	6	1	114
V 912	0	2	0	3	1	4	8	4	4	11	7	0	3	1	5	1	54
V 914	1	5	0	5	0	2	0	0	1	14	0	2	1	0	0	0	31
V 920	0	1	0	7	0	1	10	0	2	9	2	0	2	0	2	0	36
Totals	1	18	6	33	11	26	57	10	17	87	38	7	22	4	19	5	361

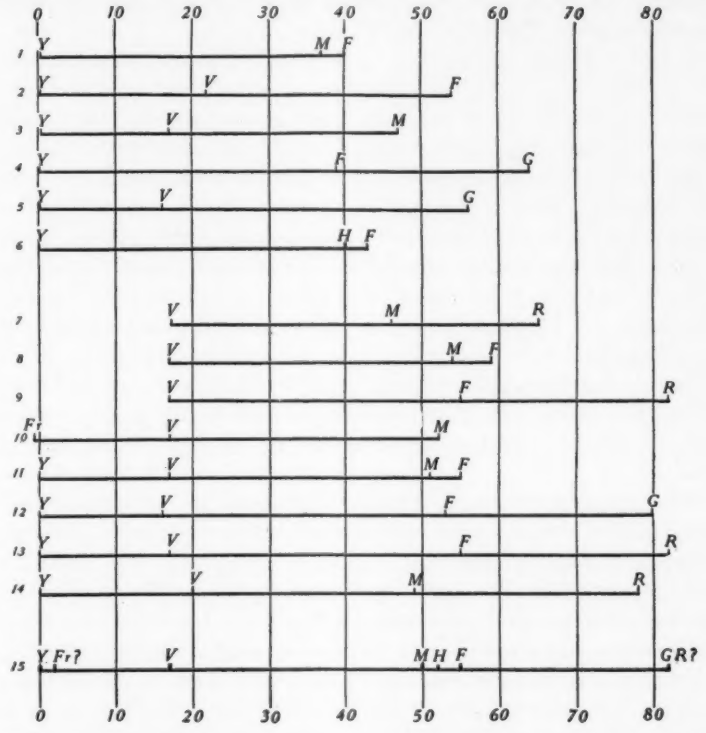
Summary of classes

Y M / V R	*M / Y V R*	*Y R / V M*	*Y V M / R*	*Y M R / V*	*Y V / M R*	*Y / V M R*	*Y V M R*
87	18	38	4	22	7	10	5
57	19	26	6	33	11	17	1
Totals 144	37	64	10	55	18	27	6

Summary of crossovers

	Y–V	*V–M*	*M–R*
	37	64	55
	10	10	18
	18	27	27
	6	6	6
Totals	71	107	106
	20%	29%	29%

might be anticipated upon the basis of preceding experiments; but they show the same general features (diagram 14).



DIAGRAMS 1-15.—The diagrams represent crossover values (percentages) in terms of distance along a straight line. Numbers 1 to 14 summarize individual experiments. Number 15 is an approximate average of the others.

The position of frayed (*Fr*) is assigned mainly on the basis of an experiment (number 1) not included in the diagrams, and may perhaps be on the wrong side of yellow. Likewise the position of rugose (*R*) is not definitely known to coincide with that of glazed, but is known to be close to it.

DISCUSSION

It is not my purpose here to enter into a detailed discussion of linkage in *D. virilis* as compared with that in *D. ampelophila*. The general re-

[5] It may be noted in this connection that in *D. virilis*, just as in *D. ampelophila*, no mutant factors have been found in the Y chromosome of the male, and no crossing over between X and Y has been detected. Cytologically the X and Y chromosomes look very much alike (METZ 1916 b).

semblance between the two is obvious from what has preceded,[5] and the work has scarcely progressed far enough to allow of more specific deductions; but there are two features brought out by the experiments that merit especial notice at this time.

The most interesting of these, in view of its possible significance, is the fact that two of the above described mutant characters in *virilis* bear a marked resemblance to two characters in *ampelophila,* and that the factors for these characters appear to occupy the same relative positions in the linkage group (chromosome). The two characters are yellow and forked. I have already called attention to the morphological resemblance they bear to their namesakes in *ampelophila* (METZ 1916 c, p. 431), and may confine myself here to a comparison of their linkage relations.

MORGAN and BRIDGES (1915) in summarizing the data on thirty-two sex-linked factors in *Drosophila ampelophila,* show that the factor for yellow is located at one extreme end of the linkage group. This they call the zero point. The factor for forked they locate 56.5 units to the right of this, or almost at the other end of the group (see their diagram 1, p. 22).

Now comparing with these the positions of yellow and forked in *virilis,* as given above, the resemblance is seen at a glance. Yellow in *virilis,* as in *ampelophila,* is at one end of the group, and forked falls about 55 units from it. The conclusion suggests itself at once that the two species correspond in respect to this much of their chromosomal organization. This particular case may, of course, be due merely to a coincidence, but if so it is remarkable that there should be such an agreement not only between the characters, but between the locations of the factors. To be sure this latter correspondence rests largely upon the terminal position of yellow in both species; and since I have studied only eight factors (sex-linked) in *virilis,* it is possible that some may be found later having loci well to the left of yellow, and removing the latter from its present terminal or sub-terminal position. On this point nothing can be said at present except that the linkage group in *virilis* is already longer (as measured by the actual amount of crossing over) than that in *ampelophila.* In *ampelophila* the sex chromosome map compiled by MORGAN and BRIDGES (1915) is only 66.2 units long, while that in *virilis,* given by the eight factors already studied, is 82 units.

Unfortunately yellow and forked are the only sex-linked characters in *virilis* that bear a striking resemblance to any in *ampelophila,* and

the comparison between the species cannot be extended to include a series of factors. Hence the evidence is by no means conclusive. I am now endeavoring to get enough additional mutants to throw more light on this point.

The second feature to which I wish to call attention is the large amount of crossing over apparent in *virilis* as compared with *ampelophila*. Whether this difference is sufficient to vitiate the comparison of linkage values given above is not clear; but in any case there is a noticeable difference as shown by the fact just mentioned, that a crossover distance of 82 units is given by the six factors yellow to glazed (leaving frayed and rugose out of account). If the 82 units represent chromosome length, then either we are dealing here with a longer chromosomal region than in *ampelophila*, or else crossing over is materially greater per unit of actual (not calculated) chromosome length. Cytologically the sex chromosomes of the two species look very much alike.[6] Those of *virilis* are perhaps a little longer, because the fly is larger, and corresponding cells (e.g., oogonia) are somewhat larger; but it is very doubtful whether this has anything to do with the difference in crossover distances.

In *ampelophila* it has been shown that linkage (crossing over) is not an absolutely fixed characteristic but may be markedly affected in different ways. For instance changes in temperature alter the amount of crossing over (PLOUGH 1917); the second brood of a female may give fewer crossovers than the first (BRIDGES 1915); and definite heritable factors have been found by STURTEVANT to lower the crossover values (MULLER 1916, p. 213). It would not be surprising, then, if a different species were found to give a different amount of crossing over, entirely irrespective of chromosome length. This I believe to be the case in *virilis*.

Judging from the data already in hand the calculated length of the distance from yellow to glazed, to say nothing of possible distances beyond them, will probably reach ninety or more units when more factors are located in this region. It is well known that the most accurate evaluation of a long distance is obtained by adding together its component short distances, and in *virilis* three long distances intervene between yellow and glazed without any available factors to break them up. These are the regions, yellow to vesiculated (17 units), vesiculated to magenta (33 units), and forked to glazed (27 units). Each of them affords

[6] Compare METZ 1916 b, plate 1, figures 2 and 19. The lower pair of chromosomes is the sex chromosome pair in figure 19.

space for numerous double crossovers, and consequently they ought to be increased by several units when more factors are studied.

It might seem at first glance that this greater frequency of crossing over in *virilis* removes the significance from the parallelism that I have just noted between the factorial loci of yellow and forked in the two species. This is not necessarily so, however. If the factors do correspond in the two species it would be expected that those in *virilis* would be more loosely linked than those in *ampelophila,* because of the greater amount of crossing over in the former. The actual values do not show this at present; but, as I have pointed out in a preceding paragraph, the calculated distance between yellow and forked in *virilis* is sure to be increased when additional factors are located in this region. It will probably become more than sixty units, so that the relative positions of the two factors may not differ greatly from those in *ampelophila.*

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COINCIDENCE OF CROSSING OVER IN *DROSOPHILA MELANOGASTER (AMPELOPHILA)*[1]

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The present investigation was undertaken in the hope of throwing some light on the behavior of the chromosomes during crossing over. The problem was considered from the point of view of genetics by studying the effect of crossing over in one region of the chromosome on crossing over in another region, and by considering how this effect varies with variation of the distance between the regions involved. The phenomena have a bearing on the method of twisting of the chromosomes and the mechanism of crossing over.

According to the theory of linear arrangement and the chiasmatype, Mendelian genes are disposed in linear series in the chromosomes, and the separation of linked genes (crossing over) is due to breaks in homologous chromosomes (which have come together during synapsis) and recombinations between the resulting pieces (JANSSENS 1909, MORGAN 1910, STURTEVANT 1913, 1915, MULLER 1916). The percentage of cases in which two linked genes separate (amount of crossing over between them) is necessarily proportional, other things being equal, to

[1] Contribution from Zoölogical Laboratory of COLUMBIA UNIVERSITY.

the distance between the genes. Hence the distance on a chromosome map is measured by this percentage of crossing over. But this percentage is also affected by other conditions than actual morphological distance; for example, the age of the female, definite Mendelian factors, etc. (STURTEVANT 1913, 1915, BRIDGES 1915, MULLER 1916). Moreover, different regions may be, and in some cases are known to be, affected to different extents. The distances on chromosome maps are, therefore, not necessarily to be interpreted as actual morphological distances, for it is possible that the amount of crossing over in the morphologically shorter of two distances is greater than in the morphologically longer. Therefore, when the term *distance* is used in this paper, it should be interpreted as *percentage of crossing over.*

Breaks may occur simultaneously at more than one point of a chromosome. For example, in the disjunction of two chromosomes of which one contains the factors *M N P Q* and the other the allelomorphic factors *m n p q* (see figure 1), when a break occurs between *M* and *N* a break may also occur between *P* and *Q*. If the pieces recombine, the resulting chromosomes will contain respectively the factors *m N P q* and *M n p Q*.

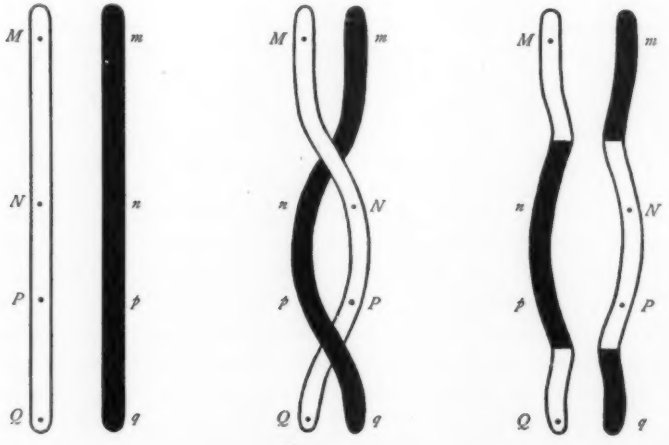

FIGURE 1

It was evident, however, from the data on which the theories above referred to were based, that when a break occurs in one region of a chromosome, neighboring regions are much less likely to undergo a break

than would otherwise be the case. If, in the above example, the regions *M N* and *P Q* are not far apart, a break between *M* and *N* will tend to prevent the occurrence of a break between *P* and *Q* (STURTEVANT 1913, 1915, MORGAN, STURTEVANT, MULLER and BRIDGES 1915, pp. 63-64, MULLER 1916).

This phenomenon, which has been termed *interference*, is well illustrated by an unpublished cross carried out by BRIDGES. The cross involved four sex-linked factors in Drosophila: vermilion (eye color), sable (body color), garnet (eye color) and forked (bristles). The relative positions of these factors are shown in the diagram (figure 2).

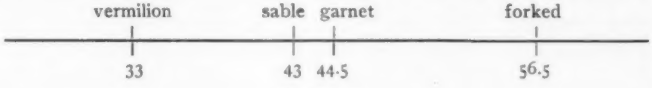

FIGURE 2

The amount of crossing over in the vermilion sable region is about 10 percent; in the garnet forked region about 12 percent. That is, if we take all the offspring from this cross, about 10 percent will be crossovers between vermilion and sable, and about 12 percent will be crossovers between garnet and forked. But if instead of taking all the flies we take only those which have already resulted from a crossing over between vermilion and sable, we find that in this sample the proportion that are also crossovers between garnet and forked is not 12 percent but only 1.2 percent. That is, these crossovers are only one-tenth as numerous as in a random sample. Or, if we count only the crossovers between garnet and forked, the number that are also crossovers between vermilion and sable is not 10 percent, but only 1.0 percent—again only one-tenth as many as in a random sample.

The actual data are as follows:[1]

TABLE 1

———	1 ———	2 ———	——— 3	1 2 ———	1 ——— 3	2 3 ———	1 2 3 ———	Total
2651	313	47	380	0	3	0	0	3394

Since the total amount of crossing over in the vermilion sable region is 0.093 and in the garnet forked region 0.113, the amount of crossing

[1] In this and other tables the non-crossover class is denoted by a line, ——— without numerals. The crossover classes are denoted by a line and numerals referring to the regions in which the crossing over took place, the regions being numbered from left to right. In the present case, the vermilion sable region is 1, the sable garnet region is 2, the garnet forked region is 3. 1 ——— indicates a crossing over in the vermilion sable region, 1 ——— 3 indicates a double crossing over involving the vermilion sable and garnet forked regions, etc. In the text, crossover classes may be indicated by the numbers of the regions in which crossing over has occurred. For example, the 1, 3 crossover class is the class involving crossing over in both the first and third regions.

over involving both regions simultaneously (if they were independent of each other) would be $0.093 \times 0.113 = 0.01$. The observed proportion of crossing over involving both regions is only 0.001. The ratio of the actual amount to the amount expected if the regions did not affect each other is $\frac{0.001}{0.01} = 0.10$. This ratio is termed the *coincidence of crossing over* of the two regions (BRIDGES 1915, MULLER 1916).[2] That is, the coincidence of crossing over of two regions is the ratio of the actual amount of crossing over involving both regions to the amount that would occur if crossing over in one region did not affect crossing over in the other.[3]

It is evident that in a double crossing over involving the regions $M\ N$ and $P\ Q$ (see figure 3), the distance between the two points at which

FIGURE 3

the breaks occur may be just greater than $N\ P$ or just less than $M\ Q$; or it may have any intermediate value. It can be shown that the average distance between the breaking points is the mean of $N\ P$ and $M\ Q$, or the distance from the mid-point of $M\ N$ to the mid-point of $P\ Q$. This was pointed out by MULLER (1916). The proof of this and a discussion of the assumptions on which it rests will be given later in this paper; I wish here merely to call attention to the formula.

COINCIDENCE OF WIDELY SEPARATED REGIONS

In the vermilion garnet sable forked cross above cited, the intermediate distance (sable garnet) is very short—only about two units. The work on Drosophila has shown that in general the coincidence of two regions increases as the distance between them increases (STURTEVANT 1915, MULLER 1916). The present investigation was undertaken to determine the value of coincidence when the intermediate distance becomes very long. Two crosses were made involving factors in the sex chromosome. The relative positions of these factors are shown in figure 4.

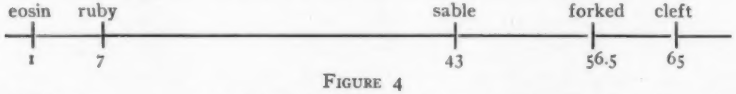

FIGURE 4

[2] This phenomenon was originally referred to in terms of interference. The index of interference used was the reciprocal of coincidence (STURTEVANT 1913, 1915, BRIDGES 1915, MULLER 1916).

[3] MULLER has preferred to state this ratio as a percentage; but for greater ease in calculation it is better expressed as a decimal fraction. The latter usage will be adopted in this paper.

One cross involved the factors eosin (eye color), ruby (eye color), sable (body color) and forked (bristles); and the other cross involved eosin, ruby, forked and cleft (venation). Practically the entire length of the sex chromosome (so far as known) is involved in the latter cross, since eosin is only one unit from the extreme left-hand end and cleft is (with the possible exception of lethal *sc*) the extreme right-hand factor. The farthest right-hand factor hitherto worked with in determinations of coincidence has been bar, which is only half a unit to the right of forked.

In choosing the factors to be worked with, it was necessary (for reasons that will be explained later) to make the regions whose coincidence was to be determined short enough to prevent the occurrence of double crossing over within them. This made the percentage of crossing over within each region small and necessitated making large counts to get significant results.

The results indicate that the coincidence of the eosin ruby region and the sable forked region (a mean intermediate distance of about 46) is about 1.00 (possibly a little more), and that the coincidence of the eosin ruby region with the forked cleft region (a mean intermediate distance of about 57) is only 0.68. That is, when the intermediate distance increases beyond a certain value, coincidence instead of rising or remaining at the value it has reached, falls again. This means that crossing over at the extreme left-hand end of the chromosome interferes with crossing over at the right-hand end, but has practically no effect on crossing over in the region just to the left of the right-hand end.

In each cross, females heterozygous for the particular combination of factors under consideration were bred singly. Since the factors involved are sex-linked, the proportion of the different classes of sons is a direct index of the frequency of crossing over in the different regions of the chromosome. The daughters were counted in order to keep track of the sex ratios, but were not classified, because it is often impossible to distinguish eosin from ruby in the female, and because it is impossible to backcross by cleft males, which are sterile. The mothers, therefore, instead of being backcrossed, were in most cases mated to bar males. Bar is a dominant sex-linked character; hence it was possible to distinguish non-disjunction in both male and female offspring (BRIDGES 1916). Cultures giving non-disjunction were excluded from the totals in both crosses, because of the possibility that non-disjunction might affect coincidence.

Each cross was made so that the factors involved entered in several different combinations in different matings. In this way any particular crossover class is represented by several different combinations of characters, so that if the viability of any particular combination of characters is poor, it may be counterbalanced by the good viability of another combination and *vice versa* (MORGAN and BRIDGES 1916). Moreover, cultures giving less than 40 males were excluded because a small number of offspring is often due to poor culture conditions, which may entail differential inviability.

The data for the eosin ruby sable forked cross are as follows:

TABLE 2

♀♀	♂♂								Total ♂♂
		1	2	3	1 2	1 3	2 3	1 2 3	
15298	6363	579	3638	1208	128	115	350	6	12387

The total proportionate amount of crossing over in the eosin ruby region is here 0.0668. The total amount of crossing over in the sable forked region is 0.1355. If crossing over in one region does not affect crossing over in the other, the amount of crossing over involving both regions simultaneously would be $0.0668 \times 0.1355 = 0.00905$. The actual amount of double crossing over involving both regions (the triple crossovers are excluded for reasons that will be explained later) is 0.00928. The coincidence is $\frac{0.00928}{0.00905} = 1.025$.[4] The two regions are therefore independent. If anything, a crossing over between eosin and ruby makes crossing over between sable and forked slightly more likely to occur.

The counts of the eosin ruby forked cleft cross are given in the following table:

TABLE 3

♀♀♂♂									Total♂♂
		1	2	3	1 2	1 3	2 3	1 2 3	
17584	6894	530	5307	828	203	47	352	14	4175

[4] The actual calculation of coincidence can be made somewhat more simply. For if the total number of flies involved is n, the total *number* of crossovers in the two regions respectively a and b, and the total *number* of double crossovers involving both regions x, then

$$\text{the coincidence} = \frac{\frac{x}{n}}{\frac{a}{n} \times \frac{b}{n}} = \frac{xn}{ab}$$

This makes it possible to calculate the coincidence of two regions without calculating their lengths.

The total amount of crossing over in the eosin ruby region is 0.0560; the amount of crossing over in the forked cleft region is 0.0875. The amount of double crossing over involving both regions (if they do not affect each other) should be $0.0560 \times 0.0875 = 0.004904$. The actual amount of double crossing over involving both regions is 0.003316. The coincidence is $\frac{0.003316}{0.004904} = 0.6761$. This indicates that crossing over in the eosin ruby region hinders crossing over in the forked cleft region.

The value obtained for the coincidence in each case is, of course, subject to a probable error of random sampling. But the applicability of the ordinary formula is doubtful in cases where the class under consideration (here the 1, 3 double crossover class), forms as small a proportion of the total as in the present case. PEARL (1917) has therefore suggested another method. DR. PEARL was kind enough to undertake a calculation of the significance of the present data, which was published in the paper referred to. This calculation, based on all the data in the eosin ruby sable forked cross, but only 9017 flies in the eosin ruby forked cleft cross, gives a probability of 0.0421; that is, the chances are about 23 to 1 against obtaining in the eosin ruby sable forked cross a coincidence as low as, or lower than, that obtained in the eosin ruby forked cleft cross.[5]

Since more data are now available, a recalculation on the basis of all the data has been made. This gives for the lower quartile of the 1,3 double crossover class in the eosin ruby sable forked cross, the value 65.3951, which is considerably higher than 47. But the probability to which this value corresponds cannot be determined from the table given by PEARL, since the latter is based on only part of the data involved.

On the Gaussian hypothesis, the observed deviation would correspond to odds of about 105 to 1 that the two values are different. But since the Gaussian method is supposed to be inapplicable to cases where p is very small and since statisticians are not in agreement on the validity of the other method (PEARSON 1917; compare also WRIGHT 1917), it seems best to suspend judgment on the statistical significance of the data.

There is, moreover, an objection to calculating the coincidence, as has just been done, on the basis of all the data lumped together. This is due to the fact that in any series of matings (such as the eosin ruby sable

[5] In the data submitted to Dr. PEARL, I did not take into account the difference between the values of the eosin ruby distance obtained in the two crosses; hence the probability arrived at by Dr. PEARL is different from that given here. This fact, of course, in no way affects the validity of the method used.

forked cross) the coincidence might be the same in all the cultures; and yet if the coincidence were calculated from the total data instead of for each culture separately, the value derived might be different from that for each separate culture, if the amount of crossing over varied in the separate bottles.

This will be evident from a simple numerical example. Let us take two imaginary cultures of 100 flies each. Let us suppose that in one culture the number of crossovers in each of the two regions involved is 10 (or 0.10 of the total) and the number of double crossovers involving both regions is 1 (or 0.01 of the total). Let us suppose that in the second culture the number of crossovers in each of the regions is 20 (or 0.20 of the total) and the number involving both regions at once is 4 (or 0.04 of the total). The coincidence in the first case is $\frac{0.01}{0.01} = 1.00$; in the second case it is $\frac{0.04}{0.04}$, which is also 1.00; but if we calculate the coincidence on both cultures together it is $\frac{\frac{5}{200}}{\frac{30}{200} \times \frac{30}{200}} = 1.11$.

We can state this in more general terms. In one culture let m be the total number of flies, a and b the number of crossovers involving respectively the two regions under consideration, and x the number of double crossovers involving both regions simultaneously. In another culture let the corresponding values be n, p and q, and y. Then the coincidence in the first case (see footnote 4, p. 140) is $\frac{xm}{ab}$ and in the second case $\frac{yn}{pq}$. The average of these two values is $\frac{1}{2}(\frac{xm}{ab} + \frac{yn}{pq}) = \frac{xmpq + ynab}{2abpq}$. The coincidence calculated on the total data is $\frac{(x+y)(m+n)}{(a+p)(b+q)}$. It is evident that these two values are not necessarily equal.

The coincidence in each cross was therefore calculated by considering each culture separately. The values obtained are given in the tables at the end of the paper. (In a few cultures there was no crossing over in

one of the two regions involved. In such cases the value of the coincidence is $\frac{0}{0}$, which is indeterminate. These cultures are consequently omitted from the calculation.)

The average value of the coincidence of eosin ruby and sable forked calculated in this way is 1.0238. The average value of the coincidence of eosin ruby and forked cleft is 0.6064. If the values are weighted according to the number of individuals in each culture, the averages are practically the same; namely, 1.0081 and 0.6049 respectively. These values agree very well with the values obtained in the previous calculation.

In table 4 the values of coincidence are grouped by intervals of 1.00, and the accompanying curves (figures 5 and 6) show these distributions graphically.

TABLE 4

Coincidence	0	1	2	3	4	5	6	7	8	9	10	Total broods
Number of broods, eosin ruby sable forked cross	91	41	12	6	2	4	1	0	0	0	0	157
Number of broods, eosin ruby forked cleft cross	141	13	7	4	3	2	1	1	0	0	1	173

It is noticeable that there is a comparatively smaller number of cultures whose coincidence is zero and a greater number of cultures whose coincidence is between 1 and 2 in the eosin ruby sable forked cross than in the eosin ruby forked cleft cross. This is partly at least due to the fact that the expected number of double crossovers involving the particular regions under consideration would (quite apart from considerations of coincidence) be greater in the former cross than in the latter, because the distance between sable and forked is greater than that between forked and cleft, and also because the eosin ruby distance in the first cross is slightly longer than in the second. However, this is counterbalanced to some extent at least by the fact that when a double crossover does occur in the second cross it raises the coincidence relatively more than in the first cross. It is in accordance with this that the highest values for coincidence are found in cultures of the second cross, not of the first, although the average coincidence in the first is higher.

Bearing in mind the possibilities of error due to these facts, we may

apply the χ^2 test (PEARSON 1911) to these two distributions. We obtain for χ^2 a value of 29.166. By referring to ELDERTON's tables (ELDERTON 1901, p. 159) we find that this value for $n = 9$ corresponds to a chance

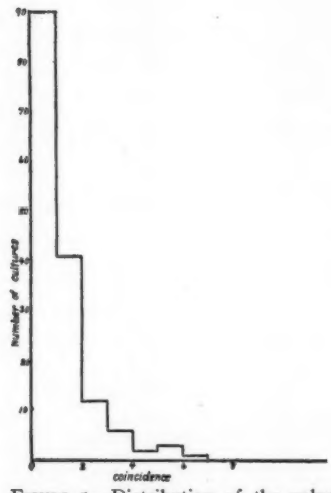

FIGURE 5.—Distribution of the values of coincidence of eosin ruby and sable forked.

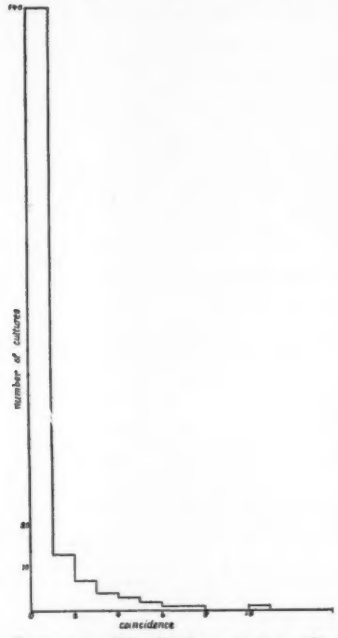

FIGURE 6.—Distribution of the values of coincidence of eosin ruby and forked cleft.

of 0.000299. Since this is equivalent to only one chance in 3344, the chance that the two distributions are the same is statistically negligible.

In some of the matings involving eosin, ruby, forked and cleft, a fifth factor, lozenge (eye), was also followed. Figure 7 indicates the location of this factor with respect to the others.

FIGURE 7

The intermediate distance between the ruby lozenge and the forked cleft regions is about the same as that between the eosin ruby and the

sable forked regions. Hence we might expect the coincidence of ruby lozenge and forked cleft to be about the same as that of eosin ruby and sable forked, and greater than that of eosin ruby and forked cleft. The actual counts in the present cross are as follows:

TABLE 5

♀♀	♂♂														Total ♂♂
	—	1	2	3	4	12	13	14	23	24	34	123	124	134	234	
3409	1217	96	321	599	164	3	31	4	53	34	44	1	1	3	1	2572

While these data are not sufficiently extensive in themselves to be significant, it may be instructive to compare them with the data already considered. The coincidence of ruby lozenge and forked cleft is here 0.866; that of eosin ruby and forked cleft is 0.295. Both values are lower than those obtained for the similar distances in the other crosses; but the disproportion between them is in the same direction and is even more marked.

A cross was also made with the factors eosin, ruby, forked and fused (wing). Since fused is between forked and cleft (figure 8), the co-

FIGURE 8

incidence of eosin ruby and forked fused might be expected to be intermediate between that of eosin ruby and sable forked and that of eosin ruby and forked cleft. The data obtained were as follows:

TABLE 6

♀♀	♂♂								Total ♂♂
	—	1	2	3	12	13	23	123	
8220	3889	290	3027	103	130	7	59	1	7506

Here the proportion of crossing over between eosin and ruby is 0.5702; between forked and fused the proportion is 0.0226. The coincidence of these two regions is 0.7221. This agrees with the expectation.

The cultures included above, as in the other crosses, were only those which contained at least 40 males, but since the number was too small to be significant another table was made in which all the other available cultures were also added in. The resulting distribution is as follows:

TABLE 7

♂♂								Total ♂♂
—	1	2	3	12	13	23	123	
4260	326	3367	118	145	10	69	3	8298

The coincidence of eosin ruby and forked fused is now 1.1144.* The

* See note on page 159.

disagreement of the calculations with each other may be due to the poor viability of the cultures containing less than 40 individuals. In any case, the small numbers involved render the results insignificant statistically.

But even if the second value obtained represents the true value of the coincidence, the result can be harmonized with that of the crosses involving eosin, ruby, sable, forked and cleft. For the coincidence of eosin ruby and forked cleft is a composite value; and the coincidence of eosin ruby and forked fused might be over 1.00 and of eosin ruby and fused cleft might be correspondingly less than 0.68. Thus the coincidence of eosin ruby and forked cleft might be 0.68, which is intermediate between the other two values. Why there should be a sudden drop of coincidence from more than 1.00 to 0.68 is hard to see. It might conceivably be connected with the fact that the fused cleft region is at the end of the chromosome. It is easy to see why the proportion of crossing over should be less in a given morphological distance at the end of a chromosome than in an equal morphological distance further in; for it might be supposed that the twisting of chromosomes at the end is not as tight as in regions further in. But since map distances are calculated on the basis of proportion of crossing over, any such decrease in the amount of crossing over must already have been taken into account in the calculation of the map distance; and it is hard to see why coincidence should be affected.

For distances less than 46, the best data for the calculation of coincidence in the sex chromosomes are MULLER'S (1916). MULLER'S curve shows a steady rise of coincidence from 0 to a little over 1.00, as the distance increases from 0 to 45. STURTEVANT'S (1915) data, which gave a value of 2.88 for the coincidence of yellow eosin and vermilion miniature (a mean intermediate distance of about 33) are too small to be significant. MULLER'S curve also shows a fall and a second rise in coincidence when the distance rises above 45; but as MULLER himself stated, this part of the curve is not significant because the data were insufficient.

We may, therefore, conclude that in the sex chromosomes of Drosophila the coincidence rises to about 1.00 as distance increases to about 46, and that coincidence declines as distance increases further.

COINCIDENCE IN THE SECOND CHROMOSOME

In the second chromosome of Drosophila, only a few determinations of coincidence have been published. STURTEVANT (1915) obtained a co-

incidence of 0.307 in a cross involving black, purple, and curved (a total map distance of about 25.5), and a coincidence of 0.599 for a cross involving black, curved and speck (a total distance of about 55) (see figure 9). The data in both cases were too small to be significant. BRIDGES (1915) obtained a coincidence of 1.11 (first broods) and of 1.00 (second broods) for black, purple and curved. This variation of coincidence with the age of the female may not be significant; but even if these figures do not represent values significantly greater than 1.00, it is evident that there must be values of coincidence greater than 1.00 in the second chromosome. For the figures obtained represent average values, including the coincidence of adjacent regions, which is generally low (less than 1.00) when the regions are short. Therefore, the coincidence of the more widely separated portions of the regions involved is probably greater than 1.00.

Dr. BRIDGES has kindly placed at my disposal the data (as yet unpublished) of a cross involving the factors star, purple, curved and speck, whose relative positions are shown in figure 9.

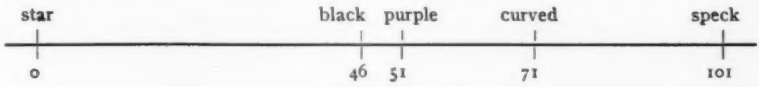

FIGURE 9

The data for this cross are as follows:

TABLE 8

	1	2	3	1 2	1 3	2 3	1 2 3	Total
1929	1487	687	1005	601	837	135	85	6766

The coincidences of the various regions are as follows:

TABLE 9

Regions	Total length	Coincidence
Purple curved and curved speck	53	0.4787
Star purple and purple curved	67	1.0226
Star purple and curved speck	99	0.9124

These figures resemble those obtained for the first chromosome in that the coincidence first rises and then falls with increase of distance, but the figures are not consistent with the figures of the black purple curved cross, which gave a coincidence of about 1.00 for a total distance of

about 20. These inconsistencies may be partly due to the variability of linkage in the second chromosome; they are probably also due to the fact that each of the regions involved (except black purple) is long enough to allow a considerable amount of double crossing over (which remains undetected) within it. It should be recalled in this connection that double crossing over for a given distance is more frequent in the second chromosome than in the first.

COINCIDENCE IN THE THIRD CHROMOSOME

The only published third chromosome data from which coincidence can be calculated are those of MULLER (1916). The counts are, however, very small. Some unpublished crosses made by MULLER and BRIDGES give results somewhat more extensive, but still too small to be significant. The coincidence does not rise much above 1.00, except in two cases in which it is over 2.00; but in both cases larger counts might change the results.

COINCIDENCE IN OTHER FORMS

In Primula ALTENBURG (1916) has obtained a coincidence of 0.64 (possibly, as he explained, this may represent a value as high as 1.00) for two adjacent regions whose lengths are 11.62 and 34.02 units respectively. The only other crosses hitherto reported involving more than two pairs of linked Mendelian genes at the same time have been carried out by GREGORY (1911) with Primula and PUNNETT (1913) with sweet peas. In PUNNETT's crosses it is not possible to calculate the coincidence, since he worked with an F_2 instead of a backcross; and GREGORY's results as reported give the linkage of only two pairs of factors at a time. Even so, coincidence could be calculated for GREGORY's data had he not been unable to follow one of the factors in all the plants. For given *AB*, *BC* and *AC* in any one cross, the doubles may be deduced (STURTEVANT 1914, BRIDGES 1914).

THE MECHANISM OF CROSSING OVER

It has been pointed out that during crossing over the chromosomes might be either loosely or tightly twisted about each other (MORGAN, STURTEVANT, MULLER and BRIDGES 1915, p. 64, MULLER 1916).

If crossing over occurs when the chromosomes are loosely twisted, i.e., when there are a few long loops, the low coincidence of crossing over of neighboring regions may be explained on the supposition that a twist in one region tends to prevent twisting in near-by regions. If the longer

loops are more frequent than the shorter ones, coincidence will rise with increase of distance between the points at which crossing over takes place. If there is a tendency to form loops of a particular length and if loops of greater or lesser length are less frequent, coincidence will rise to a maximum for an intermediate distance corresponding to the most frequent length of loop, and will then decline. The maximum coincidence might be greater than 1.00, but it might also be less. If the most frequent length of loop is sufficiently short to allow more than one to be formed in the same chromosome, there may be more than one maximum value for coincidence corresponding to the several intermediate distances. It is evident, therefore, that on the supposition of loose twisting all the known facts of coincidence may be explained.

On the other hand, let us suppose that during crossing over the chromosomes are tightly twisted; i.e., that there are many short loops instead of a few comparatively long ones. The low coincidence of crossing over of neighboring regions is then to be explained on the hypothesis that a break in one region loosens the twisting and thus prevents breaks in neighboring regions. If, however (owing to friction, adhesion or what not), more distant regions are loosened less quickly or not at all, we should expect the coincidence of widely separated regions to rise and even to reach 1.00. MULLER has pointed out that coincidence on this scheme might also rise above 1.00. MULLER's scheme could be used to explain a decline in coincidence after it had once risen above 1.00; but it is hard to see how it could explain a decline in coincidence after it had risen to only 1.00. For since a crossing over in the eosin ruby region does not affect crossing over in the sable forked region, it can not affect the coincidence of sable forked and the region to the right of forked.

If, therefore, the determinations of coincidence in this paper are valid and comparable with each other, they seem to show that *the twisting of the chromosomes during crossing over is loose; or, if it is tight, that the distance between the places of crossing over depends on other conditions than the mere tension due to the twisting.*

TRIPLE CROSSING OVER

In the above calculations of coincidence, triple crossovers were excluded from the double crossover class under consideration, in spite of the fact that the triples involve crossing over in the same regions as the doubles. Of course, coincidence might be arbitrarily defined so as to exclude the triples. While it is neither necessary nor desirable to limit the definition in this way for all cases (since the word may be applied

in any sense, provided the sense in which it is applied is stated), it should be observed that triple crossing over involves conditions different from those involved in double crossing over. For in double crossing over the intermediate region remains unbroken, while in triple crossing over the intermediate region breaks. If the chromosomes are tightly twisted at this stage, the intermediate region is loosened up in triple, but not in double crossing over. If the chromosomes are loosely twisted, a double crossing over need involve only a single loop, whereas a triple crossing over necessarily involves at least two shorter loops within the same distance, as indicated in figure 10.

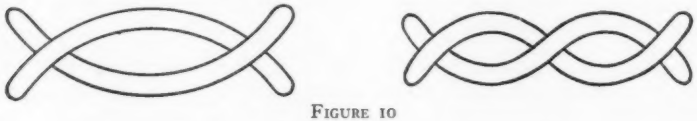

FIGURE 10

The coincidence as calculated in the data given (that is, omitting the triples from the double crossover class) measures the tendency of a second break to occur without the interposition of an intermediate break. If coincidence be calculated by including the triples in the double crossover class, it would measure the tendency of a break to occur without regard to whether or not the intermediate region remains intact. If the chromosomes twist loosely during crossing over, it is obvious that for the calculation of the most frequent length of loop the value of the coincidence should be obtained by omitting the triples from the double crossover class.

In the eosin ruby sable forked cross, the amount of triple crossing over is so small that its inclusion would make no appreciable difference. The value of the coincidence of eosin ruby and sable forked would be raised from 1.025 to 1.078. In the eosin ruby forked cleft cross, however, the coincidence would be appreciably raised, namely, from 0.676 to 0.878. This still leaves the coincidence of eosin ruby and forked cleft markedly less than that of eosin ruby and sable forked, but it suggests that in crosses involving a larger proportion of triple crossovers—as in the second chromosome of Drosophila—the inclusion of the triples in the double crossover class might disguise the results. For while in one cross the double crossovers might be significantly lower than in the other, the triple crossovers might in the first cross be sufficiently more numerous than in the second to make the coincidence (calculated by including both classes) equal in one case to that in the other. This would hide the fact

that coincidence in each case is a composite made up of two respectively different values.

Triple crossing over in the sex chromosome is comparatively rare, and only six cases have hitherto been observed: one by STURTEVANT (1915), one by MULLER (1916), and four by BRIDGES. If, as the variation of coincidence with distance suggests, the distance between the two breaking points of a double crossing over tends to be greater than half the length of the chromosome, the chromosome is not long enough to allow two such loops to occur and we should expect the percentage of triple crossing over to be low. It is in accordance with these facts that relatively more triple crossovers were obtained in the eosin ruby forked cleft cross (a total distance of 65.5) than in the eosin ruby sable forked cross (a total distance of 57).

It is possible to calculate the coincidence of triple crossing over in a manner similar to that of calculating coincidence of double crossing over. In the latter case it will be recalled the formula is $\frac{x}{ab}$, where a and b are the respective proportions of crossing over in the regions involved, and x is the proportion of double crossing over involving both regions simultaneously. In the case of triple crossing over, if a, b and c are the proportions of crossing over in the regions involved, the expected amount of triple crossing over is abc, provided the regions do not affect one another. If x is the actual proportion of triple crossing over, the coincidence is $\frac{x}{abc}$

The coincidence of triple crossing over of eosin ruby, ruby sable, and sable forked is 0.16; the coincidence of triple crossing over of eosin ruby, ruby forked and forked cleft is 0.4858. In the second chromosome the coincidence of triple crossing over in the star purple curved speck cross is 0.4157.

The phenomenon of triple crossing over raises the question of how to calculate the coincidence of distances of which at least one is sufficiently long to allow double crossing over to occur within it. Let us suppose that in the diagram (figure 11) the distance PQ is long enough to

FIGURE 11

allow double crossing over to take place within it. Should these doubles be included in the calculation of coincidence? The question is really a

matter of definition. Perhaps the simplest way would be to disregard entirely the double crossovers within PQ and to calculate crossing over between P and Q on the basis of the individuals which are only single crossovers in this region. This is mathematically self-consistent; for if the proportion of single crossing over within MN is a, and within PQ is b, the proportion of crossovers which are singles within MN and at the same time singles within PQ will be (on chance alone) ab.

But the interpretation of such calculations of coincidence may be misleading. For suppose that a crossing over in MN prevents crossing over near P. Then it would lower the amount of double crossing over within PQ, since the total distance within PQ available for double crossing over would be decreased. To look at it in a slightly different way, the occurrence of a crossover in MN would move a double crossover within PQ further to the right, so that one of the breaks involved might fall to the right of Q. This would obviously increase the frequency of single crossing over within PQ at the expense of double crossing over within the same region. Consequently, the apparent coincidence of crossing over of MN and PQ might be high; but this would mean only that crossing over within MN helps *single* crossing over within PQ, for the *total* amount of crossing over within PQ would be cut down.

This suggests that it might be best to include the doubles within PQ in the calculation. We can not always in practice do this, since there may not be an intermediate factor between P and Q which can be followed. The choice still remains, however, of counting each double as one crossing over or as two. The matter is again a question of how we choose to define coincidence.

The shortest distance in the sex chromosome within which a double crossing over has been observed to occur is 13.5. Hence, the considerations just mentioned do not call into question the validity of the calculations of coincidence of eosin ruby and forked cleft or of eosin ruby and sable forked. For the eosin ruby and forked cleft distances are too short to allow double crossing over to occur within them; and while the sable forked distance is just sufficiently long, the frequency of such double crossing over is so small (only one case has been observed in all the Drosophila work) that the result would not be appreciably affected. The ruby lozenge distance, which is 16 units long, is also too short to be appreciably affected.

In the second and third chromosomes of Drosophila the shortest distance within which double crossing over has been observed to occur is shorter than for the sex chromosome. Moreover, as has been pointed

out, the distances in the second chromosome for which coincidence has been calculated are so long as to allow a great amount of double crossing over within them. Consequently, the interpretation of these data should be attended with caution.

MAXIMUM AND MINIMUM COINCIDENCE

It may be interesting to compare the observed values of coincidence with the maximum values mathematically possible under the circumstances. The latter values can be calculated as follows:

If a and b are respectively the lengths of (proportions of crossing over within) the regions under consideration, the amount of double crossing over involving both regions simultaneously is abx, where x is the coincidence. Now, the maximum number of double crossings over will occur when every crossing over in one region is also a crossing over in the other. That is, when coincidence is at a maximum,

$$abx = a,\ x = \frac{1}{b} \text{ where } a < b$$

$$abx = a = b,\ x = \frac{1}{b} = \frac{1}{a}, \text{ where } a = b$$

That is, the greatest possible coincidence of two regions is the reciprocal of the frequency of crossing over in the greater region (if the regions are unequal), or of either region (if the regions are equal).

The greatest significant coincidence observed in the sex chromosome was 1.025, in the case of eosin ruby and sable forked. The maximum possible coincidence in this case is $\frac{1}{0.1355} = 7.3776$. The coincidence actually obtained falls far short of this.

The formula for maximum possible coincidence can be extended to any case of multiple crossing over. For example, in the case of triple crossing over, where a, b and c are the distances involved and x the coincidence, $abcx = a,\ x = \frac{1}{bc}$ where a is the shortest distance involved. If a equals b, $x = \frac{1}{bc} = \frac{1}{ac}$. If $a = b = c$, $x = \frac{1}{ab} = \frac{1}{bc} = \frac{1}{ac}$.

The minimum possible value of coincidence is obviously 0; but this is true only when the sum of the proportions of single crossing over within each of the regions involved is not greater than 1.00. For it is evident that if this sum is greater than 1.00, some crossovers in one

region must also be crossovers in the other and the coincidence must be greater than 0.

THE DISTANCE BETWEEN THE BREAKING POINTS IN DOUBLE CROSSING OVER

Given the factors $M\ N\ P\ Q$ in the order mentioned (see figure 12)

FIGURE 12

the average length of a double crossover involving the regions MN and PQ is $NP + \frac{MN}{2} + \frac{PQ}{2}$. For every such crossover necessarily includes NP, and it has an equal chance of involving in addition any length from 0 to the entire distance NM on one side, and 0 to PQ on the other. It would on the average, therefore, involve half of NM and half of PQ. This value is obviously the mean between the longest and the shortest possible distances, or it is the distance from the mid-point of NM to the mid-point of PQ. Where only three factors are involved, NP is 0; hence the value of the mean intermediate distance becomes $\frac{MN}{2} + \frac{NQ}{2} = \frac{MQ}{2}$, or one-half the total distance.

This proof rests (as has been mentioned) on the assumptions that a break is just as likely to occur at any point as at any other, and that the distance between breaks (length of a double crossing over) is no more likely to be of any particular length than would occur as the result of pure chance. The first of these assumptions is justified, since the term *distance* is used to represent the frequency of crossing over and not necessarily morphological distance. The second assumption, however, is not true in general; for, as we have seen, coincidence varies with the intermediate distance, and only for some distances (where coincidence is 1.00) are double crossovers as frequent as they would be on pure chance. Hence, in any case the true average value will be a weighted mean. Since we do not know accurately how coincidence varies with short increments of distance, we cannot judge just how accurate the formula is in any case. But if variation in coincidence is gradual, the formula must be a good index of the mean intermediate distance when MN and PQ are short.

Double crossing over within short distances is very rare, and in the sex chromosome has never been observed to occur within distances shorter than 13.5. If, therefore, in any sex-linked cross in Drosophila the intermediate distance is less than 13.5, allowance can be made in the formula for the mean intermediate distance for the absence of the

shorter double crossovers by substituting 13.5 for NP provided that $MQ > 13.5$.

When the distance NP is longer than 13.5, double crossing over may occur within it, and if no factors in this region are followed the double crossing over can not be observed. Hence the apparent amount of crossing over in the intermediate region will be less than the true value. For example, the value obtained for the distance between ruby and sable in the eosin ruby sable forked cross was 0.333; and the value obtained for the distance between ruby and forked in the eosin ruby forked cleft cross was 0.415. The map values for these regions are respectively 36.5 and 51.5.

It is, of course, possible, though unlikely, that the amount of undetected double crossing over within the ruby sable region in the first cross was sufficiently greater than the amount of double crossing over within the ruby forked region in the second cross to make the ruby sable region in the first case greater than the ruby forked region in the second case. This is further suggested as a possibility by the fact that the crossing over in the eosin ruby region is slightly greater in the first case (0.0668) than in the second (0.0560). And this might be held to explain why the coincidence of eosin ruby and forked cleft is lower than that of eosin ruby and sable forked. But even if we increase the map value of the ruby sable distance in the ratio of $\frac{0.0668}{0.0560}$, the distance will be only 41.61. This is still considerably less than the map distance of ruby forked and is almost exactly equal to the apparent length of the latter distance in the eosin ruby forked cleft cross. Since the true length in the latter case must have been greater than 41.61 because of the occurrence of (unobserved) double crossing over within it, the suggestion that the ruby sable region was genetically longer cannot be considered probable, though it remains as a rather remote possibility.

To dispose of this possibility absolutely it would be necessary either to follow enough factors in the intermediate region so that no double crossing over remains unobserved, or to make a cross involving simultaneously all the regions whose coincidences are sought The former method has the disadvantage that a great number of mutant factors tends to cause differential non-viability and that it is not always feasible to obtain properly spaced factors which can be worked together. The second method was actually tried by making crosses involving simultaneously eosin, ruby, sable, forked and cleft. But it was found that sable cleft flies were

almost always non-viable and the cross was abandoned. The method was, however, successfully used in the cross involving simultaneously eosin, ruby, lozenge, forked and cleft. Here the ruby forked distance is necessarily longer than the lozenge forked distance, since the former consists of the latter plus the ruby lozenge distance. It will be recalled that the results of this cross, while not numerically great, were in accordance with those of the two main crosses.

MUTATIONS OBSERVED

Several mutations were observed in the course of this work.

1. *Yellow body color.* Three yellow males appeared in a cross of a female carrying the factors eosin ruby forked in one sex chromosome and the factor fused in the other, by a bar male. The other offspring fell into the expected classes. Of the three yellow flies, one was also eosin fused, a second eosin ruby fused, and the third eosin forked; so that they also (except for the yellow character) fell into expected classes. This, together with the fact that the amount of crossing over between yellow and eosin is only one percent, and that no other yellow eosin flies were then, so far as known, in existence, makes it quite unlikely that the three yellow flies were the result of contamination. The yellow factor must therefore have arisen by mutation in the sex chromosome of the mother, for the mutants were all males and did not arise by non-disjunction.

The new yellow was ascertained to be sex-linked and recessive to wild, like the old yellow. When it was mated to the old yellow the daughters produced were yellow; hence the two factors must be the same.

2. *Achete.* The laboratory stock of yellow was discovered to be pure for a factor causing a reduction in number, and sometimes a total absence of, the dorso-central bristles. This factor, termed achete, is a sex-linked recessive. No crossovers between it and yellow were observed in over 200 flies; hence it is either closely linked to yellow or an effect of the yellow factor itself. If the latter turns out to be true, the yellow locus may furnish a case of quadruple allelomorphism, for another yellow stock (containing also white eyes) and the yellow which arose independently (as reported above) have the normal number of dorso-central bristles (four), and there are besides two other allelomorphs, spot and normal.

3. *Lethal.* Two females in the eosin ruby sable forked cross gave lethal ratios. The lethal in each case is about one unit from yellow; in one case it is known to be to the left of yellow. It is, therefore, the

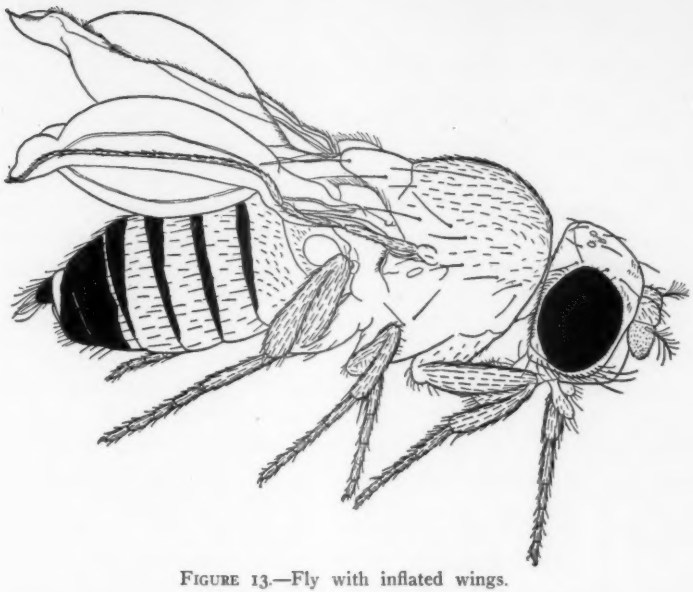

FIGURE 13.—Fly with inflated wings.

farthest to the left of any factor known in the sex chromosome. Since the females were sisters, the same factor is probably involved in both cases.

4. *Inflated.* In several of the cultures of the eosin ruby forked cleft cross there appeared flies whose wings were inflated (figure 13). As the flies grow older the wings collapse and look blistered. This variation was ascertained to be sex-linked and to be located about 1 or 2 units to the left of forked, in approximately the same locus as the factor for rudimentary wing. But the two factors are not allelomorphic to each other, since the F_1 females of the cross between them are long-winged.

In several of the cases reported above, more than one individual displaying the same mutant character appeared in the same brood. It seems unlikely that the character arose independently in each individual; more probably the individuals in each case were derived from a single mutated germ cell. If this is true, the yellow mutation must have occurred at least before the next to the last oögonial division, since it arose in the female; the lethal factor must have arisen at least before the first maturation division if it occurred in the mother of the females tested, but it may have originated between the first and second maturation divisions if it arose in the father.

SUMMARY

It has been known that the coincidence of crossing over of two regions increases in general as the distance between them increases. The evidence presented in this paper indicates that, for the sex chromosome of *Drosophila melanogaster*, when the intermediate region reaches a value of about 46, coincidence is approximately 1.00; and as the intermediate distance increases still further, coincidence decreases again. In other words, crossing over in one region of the chromosome interferes with crossing over in neighboring regions. But this influence decreases as the distance between the regions increases, until when the distance is about 46 units the interference has practically disappeared. For regions more than 46 units apart, interference reappears again.

It is pointed out that if the data presented are statistically significant, either the twisting of the chromosomes during the process of crossing over is loose, or the distance between the places of crossing over in the chromosome is dependent on other conditions than the mere tension due to the twisting.

I wish to thank Professor T. H. MORGAN and Dr. H. J. MULLER, Dr. C. B. BRIDGES and Dr. A. H. STURTEVANT for helpful suggestions made in the course of this work. I wish also to thank Dr. RAYMOND PEARL, Dr. J. A. HARRIS, and Mr. J. W. GOWEN for help with the statistical aspects of the problem.

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NOTE ADDED IN THE PAGE PROOFS.

At the bottom of page 145 the value of the coincidence of eosin ruby and forked fused (for all the broods, including those containing less than 0 males) should be 0.8572 instead of 1.1144 as given. (The latter value represents the coincidence calculated by including the triples in the double crossover class.) The suggestion made on page 146 in connection with the value 1.1144 is rendered unnecessary, since the correct value agrees with the expectation and is consistent with the other results obtained.

TABLE 10

Individual culture counts of crosses involving eosin (w^e), ruby (r_b), sable (s), and forked (f).

(1) $\frac{w^e\ sf}{r_b}$

Culture No.	Females	Males														Total males	Coincidence of $w^e r_b$ and sf
				1		2		3		1 2		1 3		2 3		1 2 3			
		$w^e sf$	r_b	$w^e r_b$	sf	w^e	$r_b sf$	$w^e s$	$r_b f$	$w^e r_b sf$	$+$*	$w^e r_b f$	s	$w^e f$	$r_b s$	$w^e r_b s$	f		
125	110	29	22	3		18	11	2	2		1			1				89	0
126	98	28	29	2	1	19	14	8	9					1	3			114	0
127	49	13	12	3	1	15	11	1	4			1		1				62	1.7714
195	89	15	15	4	1	18	9	3	1	1	1			1	2			71	0
200	100	16	23	2	2	15	10	1	3			2						74	4.1111
225	68	13	17	1	1	13	1		3				1					50	4.1667
226	100	20	36	2		12	6	4	5		1	1		3	2			92	1.5333
227	91	17	26	1	1	11	4	2	4			1		2				69	2.5556
228	104	12	26	1	3	17	5	1	4		1			2				72	0
241	88	19	17	4	4	12	7	1	1					3		1		69	0
196	64	16	10	1	2	17	7	2	1					1	2			59	0
198	71	16	8		1	10	10		4					2	1			52	0
203	77	14	18		2	10	6	3	5		1			1				60	0
224	96	15	13	1	3	12	3	1	5		1		1	3				58	0.9667
229	100	23	19	3	4	21	11	1	5	2	2	1	1	4				97	1.2436
231	83	10	12	4	2	11	11	2	1				1					54	1.9286
233	87	13	13	2	3	17	9	4	5	1	1		1	1	1			71	0.7396
234	90	19	18	1	3	17	11	1	7						2			79	0
236	76	14	17	2	2	16	6	5	4	1					2			69	0
230	105	19	29	3	1	14	7	5	4			1						83	1.6600
240	97	27	22	7	4	15	15	7			2			1	1			101	0
245	74	21	12	2	2	9	10		4					1	1			62	0
246	75	13	13	3	1	13	5	1	1		1	1		1	1			54	1.8000
128	91	20	21	1	1	13	16	4	1		1	1		2	1			82	2.2778
104	71	13	17	1	1	9	2	2	4									49	0
202	70	7	15	2	1	8	5	1	1									40	0
137	88	18	22	2		18	10	3	5	1	1			1	3		1	85	0

* The symbol $+$ indicates *wild type*.

TABLE 10 (continued)

(1) $\frac{w^e \quad sf}{r_b}$ (continued)

Culture No.	Females	Males																Total males	Coincidence of $w^e r_b$ and sf
		—		1		2		3		1 2		1 3		2 3		1 2 3			
		$w^e sf$	r_b	$w^e r_b$	sf	w^e	$r_b sf$	$w^e s$	$r_b f$	$w^e r_b sf$	+	$w^e r_b f$	s	$w^e f$	$r_b s$	$w^e r_b s$	f		
138	70	11	18		1	12	11	2	4		1	1	1					62	3.8750
141	101	26	24	3		18	11	1	4	1		1		1	2			92	2.0444
263	88	16	14	3		13	6	2	1	2	2							59	0
267	86	15	24	2	3	10	8	4	5			2	1	5	2			81	1.5987
269	56	14	12	2	1	11	7	3	5						2			57	0
270	104	16	29	2	3	16	15	3	4					1				89	0
275	90	6	22		2	10	5	2	6		3			3				59	0
271	86	11	30	2	5	20	11	1	2	1				2				85	0
273	92	14	21	2	1	15	9	3	3	1	2	1			1			73	1.3036
129	123	21	23		3	18	9	3	2						1			80	0
132	90	14	20	1	2	24	11	4	2		1			2	2			83	0
133	50	24	26	3	1	15	6	4	4				1	1	3			88	1.3538
134	144	29	10	3	2	19	20	8	6	3	4	1	1	3	2			120	0.8163
135	123	20	18	3	2	21	13	4	3		1	1	1	2	1			90	1.8750
136	96	27	23	2	1	18	8	9	6			2		2	1			99	1.9800
144	120	23	20	1	1	23	13	3	4	1		1	2					101	5.0500
264	112	22	18	1	2	19	11	5	4	4					1			87	0
265	95	13	17		7	11	13		4	2				2				69	0
262	106	22	22	2	1	16	4	4	4	1		1		1				78	1.5600
146	91	12	18	4	5	13	14	4	1				1	4	1			77	0.70000
145	101	10	23	1	2	11	8	2	3	1	1		1	3				75	1.3889
295	67	18	11	3	1	14	12	1	5	1			1					67	1.5952
297	107	22	25	1	5	16	16	2	6	1	1	1	2		5			103	1.7557
336	87	15	24	4	2	9	12	3	5		1	1	1	1				78	1.5758
130	130	14	18	2	2	28	13	3	6				1	1				88	1.6000
Totals	4726	904	1030	105	102	780	488	145	192	25	31	22	19	66	46	1	1	3957	

TABLE 10 (continued)

(2) $\frac{w^{c}r_{b}\ f}{s}$

Culture No.	Females	Males																Total males	Coincidence of $w^{c}r_{b}$ and sf
				1		2		3		1 2		1 3		2 3		1 2 3			
		$w^{c}r_{b}f$	s	$w^{c}s$	$r_{b}f$	$w^{c}r_{b}s$	f	$w^{c}r_{b}$	sf	$w^{c}f$	$r_{b}s$	$w^{c}sf$	r_{b}	$w^{c}r_{b}sf$	+	w^{c}	$r_{b}sf$		
213	98	26	23		4	8	7	5	3	2			2	2	1			83	1.5962
214	110	22	11	3	1	15	10	4	5	1	1		2	1	1			77	1.4808
215	108	24	23		1	4	10	7	5	1		2		1	1			79	2.4687
216	134	12	22	2	2	11	14	4	6		1			1				75	0
218	92	15	17	5	1	7	7	2	3	1				1	1			60	0
219	107	29	17		3	11	16	1	1				1		1			80	5.0000
250	87	18	18	2	1	3	17	6		2				4	4			75	0
302	155	45	40	2	2	17	21	8	5					3				143	0
303	149	49	28	2	1	12	9	5	7	1		1			1			116	1.9333
306	147	33	36	4		11	20	7	6	1		1		1				120	1.3333
310	134	40	24	3	1	12	18	2	5	1				1				107	0
323	105	30	23		3	9	14	3						2	1			85	0
223	118	20	12		3	10	15	5	6		1	2	1		1			76	2.1714
305	122	33	31	3		10	12	9	6				1	1	1			107	0.1486
208	103	23	21	3	4	11	13	6	2			1	1		1			86	1.7374
210	70	23	14			5	15	3	3	1								64	0
211	105	15	21		1	3	14	3					1		1			59	5.9000
212	88	26	19	1	3	8	17	3	8		1	1		4	2			93	0.8611
220	104	18	22	2	1	13	9	2	2		1	1		1	1			73	2.0857
221	62	16	17	1	2	8	11	4	9	1	2				3			74	0
222	85	19	16	3	3	17	14	5	5		1	2			2			87	1.3810
251	126	28	25	2	7	17	24	7	3		2			4	1			120	0
252	81	15	10	2	3	6	7		4	1	2		1	1				52	0.9630
253	70	15	13	2	1	6	12	5	2			1		1				58	1.6111
254	135	27	21	1	5	14	22	15	7			1	1	1	2			117	1.0833
256	135	21	23	5	3	14	11	5	8		1			3	3			97	0
257	58	17	10	1	1	12	10	3	1					2	1			58	0

TABLE 10 (continued)

(2) $\frac{w^e r_b \quad f}{s}$

Culture No.	Females	Males																Total males	Coincidence of $w^e r_b$ and sf
				1		2		3		1 2		1 3		2 3		1 2 3			
		$w^e r_b f$	s	$w^e s$	$r_b f$	$w^e r_b s$	f	$w^e r_b$	sf	$w^e f$	$r_b s$	$w^e sf$	r_b	$w^e r_b sf$	+	w^e	$r_b sf$		
259	93	21	24	2	4	13	13	4	4		1	1		3	1			91	0.8750
260	64	18	15	1	1	12	11	4	2				1	3	2			70	1.9444
261	83	17	23	3		11	14	3	4	1				1	2			79	0
301	160	41	32	5	1	23	25	10	11				1	1	3			153	0.8407
309	149	27	24		2	18	20	12	4			1	2	1	1			112	3.2000
311	120	29	9	1	1	12	16	6	3	2				2	1			82	0
312	99	22	23	1	1	13	4	7	4				1	1	5			82	1.5850
314	112	33	23	1		4	19	4	5		1		1		3			94	2.4103
315	116	42	31	1	3	10	18	10	8					1				124	0
317	77	33	24		1	5	4	6	4									77	0
318	96	20	30		2	8	11	6	4		1							82	0
319	52	12	12	1	1	8	7	2	4					2	1			50	0
320	103	17	43	2	1	11	16	4	7						2			103	0
321	85	21	15	2	1	6	11	2	4	1			1					64	1.8286
322	102	25	28	2	4	16	18	8	3					1	1			106	0
324	114	27	25	3	6	9	10	5	2		1			1	2			91	0
325	105	26	29	1	1	18	20	2	10				1	1	3			112	2.1961
316	66	21	10	1	2	3	7	1	2	1								48	0
282	70	19	14	1	4	8	18	3	4	1	1			1				74	0
283	90	12	13	1	2	5	9	2	1	1			1	1	3			51	1.2750
284	84	12	19	1	3	4	7	1	4				1	2				54	1.3500
285	75	12	10	3	1	11	15	9	6	2	1		1		2	1		74	0.4328
286	110	26	13	3	4	14	17	4	6		1	1		1	6			96	0.5926
287	71	14	15	2	3	8	11	2	3	1			1	1	1			62	1.1071
292	82	20	20	4	2	16	12	3	2				1	2	1			83	1.3175
357	78	15	19		1	3	7	1	5				1					52	3.7143
358	82	10	20	1	3	3	11	4	3	1				1	1			58	0

TABLE 10 (continued)

(2) $\frac{w^{e}r_{b}\ f}{s}$ (continued)

Culture No.	Females	Males																Total males	Coincidence of $w^{e}r_{b}$ and sf
		—		1		2		3		1 2		1 3		2 3		1 2 3			
		$w^{e}r_{b}f$	s	$w^{e}s$	$r_{b}f$	$w^{e}r_{b}s$	f	$w^{e}r_{b}$	sf	$w^{e}f$	$r_{b}s$	$w^{e}sf$	r_{b}	$w^{e}r_{b}sf$	+	w^{e}	$r_{b}sf$		
359	77	19	13	1		6	9	3	3				1	1	2			58	2.90000
395	107	28	31	5		9	15	4	3	1				2	1			99	0
405	100	24	26		4	9	16	8	3						2			92	0
288	72	11	10	1	2	5	8	2	1		1			1	1			43	0
352	78	11	15			1	4	3	4						2			40	0/0
330	134	20	30	6	1	20	16	12	6	1	2	2		3	1			120	0.8333
331	116	35	32		2	12	18	3	3						2			107	0
332	145	21	27	2	1	12	18	6	3		1	1		1	1			94	1.5667
334	134	28	41	3	1	17	22	3	9					3	4			131	0
337	83	20	23		1	2	10	5	1									62	0
338	64	16	11		3	2	14	5	1	1								53	0
339	112	30	29	2	2	8	22	8	8					2	1			112	0
340	69	10	9	1		5	10	11	3				3					52	2.2941
341	93	28	21	1	6	9	13	3	7					1	3			92	0
342	76	21	16	2	2	2	10	3	2						1			59	0
343	77	14	22	1		12	10	3	1			2		2	2		1	70	3.1818
345	95	29	19	3	3	11	6	4	5	1		2	1	2	1			87	1.7400
346	111	22	19	3	1	5	7	7	3						3			70	0
347	83	16	30	3	2	4	17	8	1	1	1			2	1			86	0
348	111	27	23	3		4	20	5	2									84	0
349	76	20	7	2	2	4	12	6	5	1			1					60	0.8333
350	103	21	19		1	8	20	5	1	1					1			77	0
353	97	24	21			7	12	7	3				1		1			76	6.3333
355	123	21	26	1		3	22	3	3						1			80	0
360	119	27	24		3	8	15	5	4					3	1			90	0
368	141	27	29	1	1	3	12	4	7									84	0
Totals	8022	1821	1693	133	150	734	1078	390	323	31	25	23	32	85	103	1	1	6623	

TABLE 10 (continued)

(3) $\frac{w^{e}\ \ s}{r_{b}\ \ f}$

Culture No.	Females	Males																Total males	Coincidence of $w^{e}r_{b}$ and sf
		—		1		2		3		1 2		1 3		2 3		1 2 3			
		$w^{e}s$	$r_{b}f$	$w^{e}r_{b}f$	s	$w^{e}f$	$r_{b}s$	$w^{e}sf$	r_{b}	$w^{e}r_{b}s$	f	$w^{e}r_{b}$	sf	w^{e}	$r_{b}sf$	$w^{e}r_{b}sf$	+		
328	108	12	26	3	3	10	7	4		2			1	6	6			80	0.5229
329	88	23	27	1	1	15	17	5	4			1		2				96	2.6667
329a	92	9	12	2	2	13	9	1	4						1			53	0
329b	67	15	17	3	3	9	10	2	1						3			63	0
377	139	31	31	2	2	19	19	1	8		1		1	1	1			117	1.6250
383	88	23	19	3	1	15	13	6	1									81	0
384	71	8	13	3	3	15	5	2	1			1	1	1	1			54	1.9286
386	141	28	31	3	1	19	12	3	6		2	1		1	1			108	1.2857
390	72	16	23	4	2	24	11	5	3	2		3	1		3	1		98	1.8846
393a	102	6	30	2		12	1	2	2			1	1	3				60	3.3333
369	72	14	11	4	1	7	4	1	3					1				46	0
371	87	6	21	1		8	3	3	1					2	1			46	0
385	101	22	1	4		15		5				1						48	1.6000
391	72	0	19			9	3	1	1									42	0/0
393c	75	8	13	2	1	11	3		2		2				2		1	45	0
389	108	15	34	2	1	12	12	2	5	1		1		3	1			89	1.4833
382	152	25	24	2	4	19	12	5	7		2		1	1				102	0.8095
370	102	3	32	2		17	1	1	2		2			1				61	0
379	171	31		3		23		1				1		1				60	5.0000
Totals	1908	304	384	46	25	272	142	50	51	5	9	10	6	23	20	1	1	1349	

TABLE 10 (continued)

(4) $\underline{w^{c}r_{b}sf}$

Culture No.	Females	Males														Total males	Coincidence of $w^{c}r_{b}$ and sf
				1		2		3		1 2		1 3		2 3		1 2 3			
		$w^{c}r_{b}sf$	+	w^{c}	$r_{b}sf$	$w^{c}r_{b}$	sf	$w^{c}r_{b}s$	f	$w^{c}sf$	r_{b}	$w^{c}f$	$r_{b}s$	$w^{c}r_{b}f$	s	$w^{c}s$	$r_{b}f$		
148	116	18	22		3	17	17	4	6			1		2				90	1.7308
151	99	19	25	4		15	13	5	13					3				97	0
147	77	6	18	1	1	8	6		7									47	0
149	87		23	3		5	7		3	1	1							43	0
150	77	7	13	1		10	5	1	2					1				40	0
152	82	5	23	1		7	6	2	4			1						49	3.5000
153	53	9	20	2	1	6	6		3									47	0
154	51	6	13	1		8	8	1	6			1			1			45	2.5000
Totals	642	70	157	13	5	76	68	13	44	1	1	3		6	1			458	

TABLE II

Individual culture counts of crosses involving eosin (w^e), ruby (r_b), forked (f) and cleft (c_t).

(1) $\frac{w^e r_b f}{c_t}$

Culture No.	Females	Males																Total males	Coincidence of $w^e r_b$ and fc_t
		—		1		2		3		1 2		1 3		2 3		1 2 3			
		$w^e r_b f$	c_t	$w^e c_t$	$r_b f$	$w^e r_b c_t$	f	$w^e r_b f c_t$	+	$w^e f$	$r_b c_t$	w^e	$r_b f c_t$	$w^e r_b$	$f c_t$	$w^e f c_t$	r_b		
4	160	37	37	2	3	22	25	1	4		1			1	1			134	0
5	95	23	23	1		19	12	1										79	0
7	98	21	21		3	12	19	5	1	1				3	1			87	0
9	108	30	20		1	15	22	1	5					2				96	0
10	97	22	15		1	13	15	3	2			1						72	6.0000
12	55	12	10	2	3	7	6	1	7	1	1			1				51	0
16	80	24	10	1	1	12	19	1	1		1							70	0
17	106	20	10		3	10	17	3	4	1	2	2		1				73	1.8250
18	79	21	14	3	1	6	18	1						1	1			66	0
19	82	9	11	1	6	8	11	3	2	2	1							54	0
20	81	25	12	2		9	22	1	1					1				73	0
21	175	34	15		1	20	28	1	5	2		1						107	3.8214
22	84	18	13	1		8	17	3	3					2				65	0
24	146	31	23	3	1	17	24	1	4	1	1				1			107	0
25	119	41	22	2	1	10	33	2	7	2				1	1			122	0
32	63	19	11		2	10	15	3	2					1				63	0
33	58	23	14	1		9	10	3	1									61	0
34	106	20	22	2	3	6	10	1	2	1		1		1	1			70	1.6667
35	96	15	23	3	2	16	14		3	3	1			1	1			82	0
36	83	12	21	1	2	12	10	1	3	1					1			64	0
54	87	16	21			10	0		5						1			71	0/0
55	91	22	18	1	3	16	8	1						2	1			72	0
56	80	21	14	1		7	21	2	2	1								69	0
57	96	30	18	1		15	17	1	6			1						89	5.5625
60	120	20	15		2	6	22	1	3					1				70	0
65	87	18	6	1	2	6	22		2					1				58	0
73	89	23	15		2	16	13		2	1				1				73	0
74	92	10	21	2	3	0	10		1	1	1				1			77	0

TABLE II (continued)

(1) $\frac{w^e r_b f}{c_t}$ (continued)

Culture No.	Females	Males																Total males	Coincidence of $w^e r_b$ and $f c_t$
		—		1		2		3		1 2		1 3		2 3		1 2 3			
		$w^e r_b f$	c_t	$w^e c_t$	$r_b f$	$w^e r_b c_t$	f	$w^e r_b f c_t$	+	$w^e f$	$r_b c_t$	w^e	$r_b f c_t$	$w^e r_b$	$f c_t$	$w^e f c_t$	r_b		
75	117	33	25		1	14	19	5	3					1	1			102	0
8	103	29	15		2	18	23		1	2				1				91	0
11	83	13	9	2	2	11	10		1	2	1			2	1			54	0
69	104	21	14	4	2	18	16		2	1				3	1			82	0
70	68	17	13	1		14	7		2	1	2	1		2				60	2.4000
23	124	27	20	3	3	16	14	3	1		3							90	0
37	66	24	18	1		8	10	1	3					1	1			67	0
39	78	14	14			6	16	1	1	1				1	1			55	0
40	100	26	27	1	2	21	14	1	4		1			1	1			99	0
48	55	25	12	1		12	12	3	2					1	1			69	0
49	90	20	15	2	2	4	16	2	1					1				63	0
51	78	20	18		1	8	18	2	2	1			1					71	4.7333
52	48	12	12	1		10	20							1				56	0
53	37	7	7	1		11	13		1									40	0
59	70	26	13			1	11	1	3					3				58	0/0
62	70	16	10	1	1	10	11	1	2	1								53	0
63	69	12	13	1		12	7	1		2	1				1			50	0
67	77	21	11			7	14	3	2					1				59	0/0
77	62	9	7		1	15	20	1	1					2				56	0
78	85	17	9		2	7	11		4									50	0
81	102	20	18	2		17	26		2	2				3				90	0
82	93	16	13	2	1	11	16	6	7	4		1		5	2			84	0.5000
84	106	27	15	3	2	12	15	3		1	1			4	2			85	0
86	94	30	18		2	14	17	3	5		1	1		4	3			98	1.5312
87	129	26	33	2	4	21	24	2	4	6	1		2	2	1			128	1.5515
88	94	23	26	2	1	13	13	2	1				1	2	1			85	3.0357
89	85	20	15	1	1	15	24	3						1				80	0
90	60	17	7		2	8	20		3				2					59	5.0000

TABLE II (continued)

(1) $\frac{w^{e}r_{b}f}{c_{t}}$ (continued)

Culture No.	Females	Males																Total males	Coincidence of $w^{e}r_{b}$ and fc_{t}
				1		2		3		1 2		1 3		2 3		1 2 3			
		$w^{e}r_{b}f$	c_{t}	$w^{e}c_{t}$	$r_{b}f$	$w^{e}r_{b}c_{t}$	f	$w^{e}r_{b}fc_{t}$	+	$w^{e}f$	$r_{b}c_{t}$	w^{e}	$r_{b}fc_{t}$	$w^{e}r_{b}$	fc_{t}	$w^{e}fc_{t}$	r_{b}		
91	112	27	29	3	2	20	20	3	7	5	1			3	2	1		123	0
94	85	21	29	1	2	20	16	3			1				2			95	0
95	142	34	23	2	2	23	28	2	5	2	3			1	3	1		129	0
96	94	23	20	1		13	20	1	1	4		1		2				86	2.8667
97	156	35	32	6	5	37	23		4	1	1				2			146	0
98	117	18	20	3	1	15	20	1		1	1				2			82	0
99	122	36	30	1		27	22	3	3	1					3			126	0
101	100	24	24	2	3	17	18	4	2	1	1		1	2	2			101	1.1477
102	79	16	8	1	2	9	11	1	1					2	1			52	0
104	84	10	16	1		13	11							2				53	0
105	82	16	14	4	3	13	17	4	3	1	1			4	1			81	0
109	104	19	10	1	1	10	11	1	5					1				59	0
110	68	19	14	1	1	8	13	2	2						2			62	0
112	75	19	17	1		18	13	1	1									70	0
113	70	20	17	3	2	5	11		4	1	1	1			1			66	1.3750
114	100	22	10	2	1	20	13		1	1					1			71	0
115	76	16	15	1	2	16	17	2	3	2				1				75	0
116	82	16	23	4		10	14		1	1					1		1	71	0
117	81	20	13	2	4	15	9	6	1	1	3				1		1	76	0
118	82	25	11	1	2	9	9		2									59	0
120	77	17	20	3	1	14	14	1	1				1					72	4.8000
122	99	20	23	1	3	16	19	1	2						2			87	0
123	62	14	20	4		10	16	1	3					1				69	0
2388[7]	113	27	20		1	20	15	2	2	1	1							89	0
2389	124	33	20	6	3	14	21	3	4	2				5	1			112	0
2390	133	31	22	1	2	22	25	3	4						1			111	0
2462	169	43	27	1	5	23	23	4	3	2	2			2	1			136	0
2463	152	42	32	3	4	30	27	8	3	2				2	5			158	0
2468	85	27	19			11	17	4	3				2	2				83	7.5455
2469	135	39	23	1	3	18	31	3	3					1				124	0

[7] Cultures 2388-2505 are from crosses made by BRIDGES.

TABLE 11 (continued)

(1) $\frac{w^{c}r_{b}f}{c_{t}}$ (continued)

Culture No.	Females	Males																Total males	Coincidence of $w^{c}r_{b}$ and fc_{t}
				1		2		3		1 2		1 3		2 3		1 2 3			
		$w^{c}r_{b}f$	c_{t}	$w^{c}c_{t}$	$r_{b}f$	$w^{c}r_{b}c_{t}$	f	$w^{c}r_{b}fc_{t}$	+	$w^{c}f$	$r_{b}c_{t}$	w^{c}	$r_{b}fc_{t}$	$w^{c}r_{b}$	fc_{t}	$w^{c}fc_{t}$	r_{b}		
2503	137	28	31	2		14	18		3					1				97	0
2504	93	21	23	1	2	11	23	2	2									88	0
2505	156	30	28	2	4	19	20	2	3	1			1	2				112	1.7500
506	59	16	8		2	6	15	1	2					1	1			52	0
508	63	11	7	1	3	12	21		2									57	0
510	84	18	8	2	1	11	11	1	3			1						56	2.8000
513	76	14	15		1	13	15	1	4		1			2				66	0
532	86	12	14	2	1	17	15	2	2	1					1			67	0
533	75	18	6	3	3	6	14	2	2		1	1			1			57	1.1875
534	109	23	19	4		13	18	3	5	1	2			3				91	0
535	85	20	18		1	6	21	3	5					1				75	0
536	98	16	19	1	1	15	18		3	1	1			1	1			77	0
537	110	28	21		1	14	24	1	8		1			1	2			101	0
538	106	28	15	1	2	13	22	3	3	1	1				2			91	0
539	88	25	13		2	12	20	1	4					1	1			79	0
540	92	19	15	1	1	9	9	1	3	1				1	2			62	0
542	59	14	9			12	12	1	2									50	0/0
543	89	12	12	1	1	1	17	1	2					1	2			50	0
545	75	19	21	1		8	12	2	1					1	2			67	0
547	57	13	13	1		6	19	1	5	1			1	1	1			62	0
503	39	12	8		1	8	6	1	2	1				1				40	0
511	65	8	14	1	1	6	11	1			1	1			2			46	2.8750
512	64	11	7	2		10	11	1	3						2			47	0
546	59	17	7			9	5	1	1		1							41	0
548	65	10	10			5	13		1					2				41	0/0
562	60	20	14	2	1	10	14	1		1				1				64	0
567	61	21	6	2	1	6	11	1	3				1					52	2.6000
Totals	10330	2406	1884	153	163	1440	1854	183	284	80	45	14	13	116	82	2	2	8721	

TABLE II (continued)

(2) $\frac{w^e \quad f}{r_b \quad c_t}$

Culture No.	Females	Males														Total males	Coincidence of $w^e r_b$ and fc_t
		—		1		2		3		1 2		1 3		2 3		1 2 3			
		$w^e f$	$r_b c_t$	$w^e r_b c_t$	f	$w^e c_t$	$r_b f$	$w^e f c_t$	r_b	$w^e r_b f$	c_t	$w^e r_b$	$f c_t$	w^e	$r_b f c_t$	$w^e r_b f c_t$ +		
418	84	13	10	2	3	9	7	5	3						1		53	0
419	61	17	10			5	12	2	1								47	0/0
422	129	24	10	2	1	2	16	3							1		59	0
449	132	28	27	3	3	23	29	5	3		1	2		3	4	1	132	1.4667
451	53	14	5		1	3	8	1	4		2			1	1		40	0
455	65	17	6		2	13	12		2								52	0
466	71	22	6	2		5	10		1		1			2		1	50	0
Totals	595	135	74	9	10	60	94	16	14		4	2		6	7	2	433	

TABLE 11 (continued)

(3) $\frac{w^e r_b}{fc_t}$

Culture No.	Females	Males																Total males	Coincidence of $w^e r_b$ and fc_t
				1		2		3		1 2		1 3		2 3		1 2 3			
		$w^e r_b$	fc_t	$w^e fc_t$	r_b	$w^e r_b fc_t$	+	$w^e r_b c_t$	f	w^e	$r_b fc_t$	$w^e f$	$r_b c_t$	$w^e r_b f$	c_t	$w^e c_t$	$r_b f$		
579	73	18	14	2	2	11	13	5	3					3				71	0
580	109	24	12	2	3	10	16	1	3	3			1					75	1.6667
581	122	27	14	3		12	17	2		3	1			2	1		1	83	0
585	87	12	18	2	4	8	12		5	1					1			63	0
589	71	17	5		2	12	12		1						1		1	51	0
594	80	11	6		2	9	16	2	4	1		1			2			54	1.5000
604	76	22	5		1	7	10	3	2	1	1				2			54	0
586	73	9	10	1		10	8	2	1	1		2	1					45	4.5000
587	64	9	11			7	13		1	2								43	0
610	56	8	10		1	8	10		2	1				1	1			42	0
611	91	17	1		4	3	15		4	1		1		2			1	49	0.8750
613	93	24	2		1		11	1	2					1	1			43	0
615	68	15				5	18	1	3	1	1			1				45	0
617	65	23	11		1	4	11	1	4			1			1		1	58	2.4167
618	72	19	12	1	1	7	21	1	2	1					1			66	0
620	83	15	14	1	1	12	12	2	4		1			1	1			64	0
660	68	13	8	1	1	4	18	2	3					1	1			52	0
803	120	17	5		6	4	23	1	2	2					1			61	0
Totals	1471	300	158	13	30	133	256	24	46	18	4	5	2	12	14		4	1019	

TABLE II (continued)

(4) $\frac{w^{c} r_b f c_t}{}$

Culture No.	Females	Males														Total males	Coincidence of $w^{c}r_b$ and fc_t
				1		2		3		1 2		1 3		2 3		1 2 3			
		$w^{c}r_bfc_t$	+	w^{c}	r_bfc_t	$w^{c}r_b$	fc_t	$w^{c}r_bf$	c_t	$w^{c}fc_t$	r_b	$w^{c}c_t$	r_bf	$w^{c}r_bc_t$	f	$w^{c}f$	r_bc_t		
679	77	13	11	1		11	10	1							2			49	0
680	44	4	18			14	11		4						2			53	0/0
681	106	10	28	3	2	25	15	5			1				2			91	0
683	84	5	20	2		11	11	2	1						2			54	0
684	118	11	31	5		22	22	1	1	1				1	2			97	0
686	79	16	19	2	3	19	7	3							2			71	0
687	167	24	36	3	1	20	13	3	2		1			4	1			108	0
688	59	14	14	1		13	8	2	2	3				1	1			59	0
689	93	16	25	4	1	12	14	3	3	2	2			1				83	0
690	101	23	27	1	3	20	12	3	2					2				93	0
691	90	12	24	3	1	21	11	5	7				1		1			86	1.2286
695	101	10	34	2		19	8	1	2	1					1			78	0
742	159	19	33	2	1	35	18	5	6	1	1	2	1		1			125	3.1250
750	127	23	20		4	15	13	4	3	1	1		1	2	3			90	0.9890
751	124	9	27			26	17	4	3				1					87	10.8750
752	125	17	22	2		14	12	8	2		1			1	2			81	0
753	125	20	32	5	3	28	23	2	6		2		1	2	1			125	0.9542
Totals	1779	246	421	36	19	325	225	52	44	9	9	2	5	14	23			1430	

TABLE II (continued)

(5) $\frac{w^{+}\ l_{z}^{+}}{r_{b}\quad f\quad c_{t}}$

Culture No.	Females	Males																										Total males	Coincidence of $w^{+}r_{b}$ and fc_{t}
		—		1		2		3		4		1 2		1 3		1 4		2 3		2 4		3 4		1 2 3		1 2 4		1 3 4		2 3 4			
		$w^{+}l_{z}$	$r_{b}fc_{t}$	$w^{+}r_{b}fc_{t}$	l_{z}	$w^{+}fc_{t}$	$r_{b}l_{z}$	$w^{+}l_{z}fc_{t}$	r_{b}	$w^{+}l_{z}c_{t}$	$r_{b}f$	$w^{+}r_{b}l_{z}$	fc_{t}	$w^{+}r_{b}$	$l_{z}fc_{t}$	$w^{+}r_{b}f$	$l_{z}c_{t}$	w^{+}	$r_{b}l_{z}fc_{t}$	$w^{+}f$	$r_{b}l_{z}c_{t}$	$w^{+}l_{z}f$	$r_{b}c_{t}$	+	$w^{+}r_{b}l_{z}c_{t}$	$l_{z}f$	$r_{b}l_{z}f$			
702	142	37	17	4	3	7	9	16	16	2	7			1		1		1	1									120	0
703	154	33	24	1	2	7	5	9	11	1	1							2	2	1		1	1					105	3.5000
704	115	20	12	1		4	9	14	16	1	4							4		1	1	1	3					91	0
705	95	13	14	2	2	3	3	8	12	1	2			1				3								1		67	0
706	161	30	29		3	9	8	13	20	4	2			1								2						121	0
743	97	31	6	2	3	4	8	8	13													1						76	0
745	82	22	17	2	1	4	9	5	10	1	3							1		1	1							77	0
797	139	36	11	2	3	7	9	1	12	4	7			1						2	1							96	0
812	194	42	19	4	3	6	13	12	24	1	7			2	3			3		2		1				1		143	0
813	148	25	26	1	4	8	2	13	12		2			3														96	0
835	116	26	11	1	1	5	3	2	12		6								1			1						69	0
836	128	28	15			8	6	9	12	4	6							1		1		2	1					93	0/0
845	80	22	17	1		3	5	9	11	1	4		1						1	1			1					77	0
890	85	18	10		4	3	2	6	13		4			1				1		1	1	1						65	0
892	67	13	11	1	1	1	2	6	10	3	2			2				1	1		1							55	0
901	125	33	19	1	1	5	12	6	19	2	4			1				2	1	3		2	2					113	0
913	148	22	22	4	6	10	12	11	21	3	6	1		3	4	2		7	2	3	1	7	2	1	1			151	0.5491
914	73	21	12	1	2	1	6	2	10		5							2		1		1	1					65	0
919	142	32	16		1	5	6	9	10		4							2		1		1						87	0
921	117	29	17			2	6	11	14	4	3							1			1	1	1					90	0/0
922	143	40	18	3	4	5	6	6	15	2	3			2					1	2		1						108	0
923	173	36	35	4	2	3	5	10	17	5	8	1								1			1					128	0
925	176	30	23	1	1	4	10	5	23	4	5			3		1		3			2		1					116	1.4872
927	137	19	27	2	3	9	3	4	14	1	7							2			2	3	3			1		100	0
928	181	26	13	2		6	8	6	12	3	4				1			2									1	84	0
943	82	14	17	1	3	3	4	7	5	2	6			1				2	3		1		1					70	0
947	139	37	22	2		8	8	11	16	1	2			1						1								109	0
Totals	3400	737	480	43	53	140	181	219	380	90	114	2	1	23	8	4		40	13	22	12	26	18	1	1	3	1	2572	

* l_{z} = lozenge.

INHERITANCE IN THE ASEXUAL REPRODUCTION OF *CENTROPYXIS ACULEATA*

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INTRODUCTION

The "pure line" concept

Since JOHANNSEN (1903), as a result of his studies on self-fertilized plants, first arrived at the conclusion that selection is ineffective within

"pure lines," the phenomenon of complete regression in such material has been shown, by a multitude of other investigators, to be of very general occurrence. It has been proved that the same general state of affairs exists, not only in self-fertilized plants, but also in plants reproducing vegetatively, by tubers or cuttings, and in animals reproducing by parthenogenesis, budding and fission. It is unnecessary to enter into the details of these investigations. I will mention only the papers of EAST (1910) on the potato, of AGAR (1914) and EWING (1914 a, 1914 b, 1916) on daphnids and aphids reproducing parthenogenetically, of HANEL (1908) and LASHLEY (1915, 1916) on Hydra, and of BARBER (1907), JENNINGS (1908) and WINSLOW and WALKER (1909) on protozoans and bacteria. All these investigators entirely corroborated the results and conclusions of JOHANNSEN.

And what are these conclusions? Simply that in organisms reproducing asexually and in homozygous organisms reproducing by self-fertilization, each species is not an ultimate unit, but is itself made up of a large number of diverse strains, called "biotypes," or "clones," differing from each other, but each one constant and invariable in its own hereditary constitution. This last idea, that every individual of any given pure line or clone is precisely the same in its genetic make-up as any other individual of the same pure line or clone, is the basic idea of the "pure line" concept. As a corollary, it follows that all the variations found within a single clone are purely somatic and not heritable. In this case, it is evident, selection working within a clone could have no effect. The testing of this hypothesis, then, is of the greatest importance for any general consideration of the mechanism of evolution.

This problem has already attracted the attention of numerous investigators. Some have completely confirmed the conclusions of JOHANNSEN. The more important of these papers have just been cited. But there are others who have maintained that hereditary variations do occur within clones, and that selection within a clone may have an effect.

PEARSON (1910), subjecting the data of the earlier pure line workers to a more accurate statistical analysis, found evidence indicating that such inheritance might exist. JOHANNSEN himself (1913) showed that sudden heritable variations (mutations) had appeared in several of his pure lines, and that these variations might be either minute or large. STOUT (1915), STOCKING (1915), MIDDLETON (1915), and JENNINGS (1916) have all succeeded in isolating, by selection, hereditarily diverse strains within single clones. The present paper adds another similar case to this list.

Problems

Accepting, as we must, the fact that there do exist, within a single species, diverse strains or races, there are two main problems which claim our attention. The main problem, undoubtedly, is the one which has already been discussed. Are "pure lines" really pure? Do heritable variations occur within the clone? It is this problem which is attacked in the present paper.

A second problem, intimately connected with the first, is presented by the very existence of diverse strains. How did these diverse strains originate? Or, to bring the problem within the horizon of the experimentalist, can new strains arise from old ones, and if so, how? The number of diverse strains which exist in nature is almost beyond belief. In my own work with Centropyxis, out of over thirty individuals, isolated from a single pipetteful of debris, taken from a single dead lotus stem, there were no two which, after a month's multiplication and careful study, could be surely assigned to the same clone. Where did these diverse races come from? How long have they been in existence? How do new races arise from existing races? To these questions we have, as yet, no answer.

Plan of the present investigation

The object of my work with Centropyxis was to find out whether heritable variations do or do not occur within a single clone. Since many investigators have reported that such variations are not to be found, it may be taken for granted that it will be no easy task to find them, even if they do occur. It is not sufficient simply to pick a character at random, and determine whether its variations are or are not hereditary. One must first show that diverse clones exist in the given species, and that these clones differ from each other in certain inherited characters. Then these characters may be studied, and those which are most favorable for showing the inheritance of variations within the clone, if it exists, may be determined. When this has been done, and not until then, one is in a position to put the question to a fair test.

There are two ways in which the inheritance of variations within the clone may be determined. The first is a statistical method, making use of the coefficient of correlation. The inheritance of diversities within the clone is always accompanied, unless some other factor intervenes, by a significant positive correlation between parents and their offspring. Also, as PEARSON (1910) has pointed out, if the size of the coefficient

of correlation diminishes as we go back to more and more remote ancestral generations, it indicates that inheritance of variations occurs within single clones. This criterion of a "diminishing ancestral correlation" applies to coefficients of correlation computed either from a single clone or from a population containing many clones.

All the more recent investigators have realized, however, that results expressed solely as coefficients of correlation are often inconclusive and hard to interpret. In particular, if an experiment extends over a considerable space of time, a gradual change in environmental conditions which affect the character studied will simulate a true inheritance of variations in the most perfect manner, so far as the resulting coefficients of correlation are concerned.

The real test for the occurrence of heritable variations within a clone must be the success or failure of an attempt to isolate, by selection, hereditarily diverse groups within a single clone.

In my own work on Centropyxis, both of these methods have been employed, and the evidence drawn from coefficients of correlation was used as a guide in the subsequent selection experiments.

In closing this brief introduction to the problem, I desire to express my deep indebtedness to Dr. H. S. JENNINGS, who suggested this problem to me, and who has greatly assisted both the experimental work and the preparation of this paper, by his kindly advice and criticism.

Material;—Centropyxis aculeata

Descriptive

The lobose rhizopod, *Centropyxis aculeata* Stein, is an excellent subject for genetic investigation. It is closely related to Difflugia, the subject of JENNINGS's recent (1916) paper, and, like Difflugia, its protoplasm is surrounded by a spine-bearing shell which fulfills all the requirements for genetic study. That is, it presents definitely measurable structural characters which are heritable, yet very variable, and which are entirely unaffected by growth or environmental influences during the life of the individual. The shape of the shell is even more favorable for study than that of Difflugia. The individuals used in this study correspond closely to the description of variety *discoides* PENARD (1902). In this form the chitinous shell, studded with sand grains and diatom tests, is decidedly flattened (see figure 2). The upper surface is convex and the lower slightly concave. Seen from above, as in figure 1, the outline of the shell is nearly circular, and the thin "anterior" edge, with its

smooth outline, is clearly distinguishable from the thick, rough "posterior" edge, which may bear from one to eleven spines, each tipped by a grain of quartz which looks like the cork in a bottle. In all but the oldest shells, the outline of the mouth, which is situated at the top of

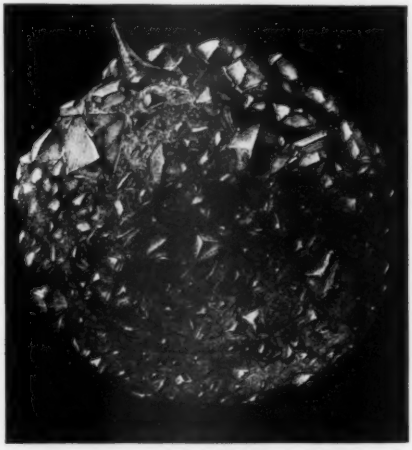


FIGURE 1.—Dorsal view of living individual of *Centropyxis aculeata.*

a funnel-like infolding of the lower surface, is plainly visible through the shell, even when the organism is viewed from the upper side.

In order to permanently record the characters of an individual, then, it was necessary only to transfer the living organism to a minute drop of water on a slide and to trace the outline of shell and spines with a camera lucida, under the high power of the compound microscope. By focussing down a little the outline of the mouth was then added. Such camera outlines were made of every individual used in the statistical part of the work, and in one of the selection experiments. Some of them are reproduced, reduced in size, as illustrations for the present paper.

The chitinous shell surrounds an ameboid protoplast. At the time of cell division the protoplast swells, probably by the absorption of water, and projects out of the mouth of the old shell. This projection assumes the size and shape of a new shell, but one with its "antero-posterior" axis in just the opposite direction to that of the old shell. To the surface of this mold flow various sand-grains and diatom tests which have been picked up by the parent organism and stored within its body. Now,

over the entire surface of the exposed protoplast, a chitinous substance is secreted, in which, as it hardens, the foreign particles become firmly embedded. After the new shell is formed, the parent protoplast is par-

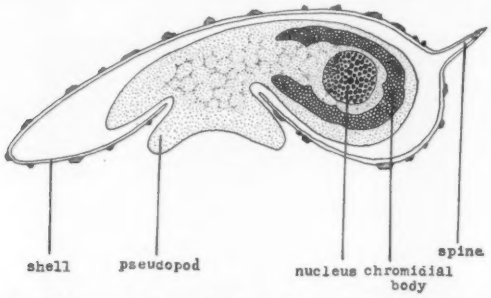


FIGURE 2.—Diagrammatic sagittal section of *Centropyxis aculeata*.

tially retracted into the old shell, the nucleus divides, and ultimately the protoplast pulls apart into two organisms, and the parent and its offspring separate.

The regular mitotic division of the nucleus during this process has been described and figured by SCHAUDINN (1903, 1911). Unfortunately, although a chromidial body is present (see figure 2), no account of its behavior during cell division has been published.

The new shell, when first formed, is almost white, in striking contrast to the dark brown color of old shells. The darker coloration is taken on gradually, first appearing as a faint yellowish tinge, which slowly deepens to brown. Even when four or five individuals have come from one in the course of a week or so, their relative ages, and therefore their parentage, is unmistakably indicated by their color. In practice, however, it was only very rarely that either of the two products of a division had time to divide again before they were separated. At ordinary laboratory temperatures, during the cooler months, Centropyxis divides about twice a week, at most. Inspection of the cultures at intervals of two or even three days was ordinarily sufficient to insure the isolation of parent and progeny before either had divided a second time.

In closing this brief description of the organism used in this experiment, I wish again to emphasize the fact that the new shell is completely produced by the parent organism before nuclear division begins. It is simply handed on to one of the products of the ensuing fission. Further, the new shell is in no sense molded on the old one. This is particularly

evident in Centropyxis, since, as figure 3 shows, the axes of old and new shells lie in exactly opposite directions. The number of spines and the shape of the shell, then, must be determined by precisely the same kind of determiners that underlie all the other hereditary characters of the species.

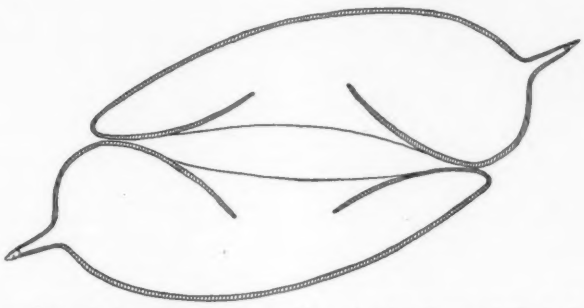


FIGURE 3.—Lateral view showing the position of old and new shells in the division of *Centropyxis aculeata.*

Previous breeding work on Centropyxis

The only other investigator who has studied the reproduction of *Centropyxis aculeata* is SCHAUDINN (1903, 1911). In his account of the life history of this species, he includes a few notes on the vegetative reproduction. Most of these statements I have been unable to confirm. He states, for instance, that the new shell is always larger than the old one. This I find not to be true. The new shell may be either slightly larger or slightly smaller than the old one. In line 30 (to be described later), with over 750 recorded fissions, the average size index of the parents was 21,969 sq. microns, that of the offspring 21,624 sq. microns. SCHAUDINN also says that he was never able to get an individual to divide more than once, and concludes that after this single fission the old individual lives on for some time and finally dies. In my cultures, individuals have repeatedly divided as many as ten times, some as many as fifteen times.

I have made no experiments on the sexual reproduction of Centropyxis, and will not discuss the life cycle. It is briefly described in SCHAUDINN'S paper (1903). I can only state that my cultures have been repeatedly interrupted, usually in January or February, by what I take to be epidemics of sexual reproduction. At the beginning of such epidemics, the protoplasts of nearly every individual in the cultures

divide into a number of small amebae, which show no trace of a formed nucleus, but contain scattered chromatinic granules. These amebae escape from the shell, leaving it empty. I was unable to follow their further history.

Methods

Cultural technique

In keeping Centropyxis in cultures in the laboratory, my aim was to keep the environmental conditions as nearly like the natural conditions as possible. Each individual was isolated in the depression of a hollow-ground slide, in about five drops of pond water. To this water was added, at the time of isolation, a few strands of the Oedogonium, in the mats of which this rhizopod was living, as well as some debris taken from among the matted filaments of the alga. This debris furnished diatoms, the principal constituent of the food of the rhizopod, as well as sand grains to be used in the construction of new shells.

In these miniature aquaria the water was changed every two or three days, and once a week all the individuals were transferred to freshly prepared slides, to avoid any possibility of an injurious accumulation of either excretion products or bacteria. Whenever a fission occurred, the new individual was removed with a capillary pipette, outlined with the camera lucida, and transferred to a new culture slide.

Records were kept on small sheets of paper, each of which contained the number of a given individual, the date upon which it was isolated, its camera outline, and the numbers and dates of isolation of all of its progeny. Later, during the process of working up the data, the spine number and certain measurements of the outline drawing were added. Since these drawings, made from the living organism while the new shell was still clean and translucent, gave all the data required, no attempt was made to preserve the shells of individuals when they died or were discarded.

In some of the later work, when attention was centred wholly on the inheritance of spine number, the records were much less elaborate, consisting merely of the number of the specimen, the date on which it was isolated, its spine number, and the corresponding data for all of its offspring.

Statistical methods and use of terms

The following measurements were taken on each of the camera outlines: Diameter of shell and mouth in the "antero-posterior" axis.

Diameter of shell and mouth in an axis at right angles to the "antero-posterior" axis. To secure a number proportional to the area of the shell, the two diameters were multiplied together. This was called the "shell size index." In order to obtain a number related in some way to the form of the shell, the "antero-posterior" diameter was divided by the other diameter. This was called the "shell form ratio." The "mouth size index" and "mouth form ratio" were computed in the same way. All measurements were made in microns, giving the actual dimensions of the shell.

The coefficients of correlation given in this paper were computed by the improved methods devised by Dr. JENNINGS (see especially JENNINGS 1916, p. 416). So many of these constants are given that it seems inadvisable to attempt to publish even all of the correlation tables, much less the extensive pedigrees on which they are based. A few of the more important tables are included in the appendix.

A word as to terminology: In this paper, following the usage of SHULL (1912) and JOHANNSEN (1913), the term "pure line" will be considered as restricted to homozygous organisms reproducing by self-fertilization. The various strains of Centropyxis considered in this paper are properly to be spoken of as "clones," that is, families arising from a single ancestor by asexual reproduction. The more general terms "strains," "lines" and "races" are considered as synonymous and as having no specific meaning.

INHERITANCE WITHIN A POPULATION

Existence of diverse clones

The first requisite, in any study of inheritance within the asexual progeny of a single individual, is to be sure that the diverse clones, whose existence we assume in our population, really exist there. Next one must demonstrate that there exist between different clones hereditary diversities in the characters which are to be studied. Only too often (in the recent papers of EWING (1914 a, 1914 b, 1916), for example) this precaution has been neglected.

My first cultures of *Centropyxis aculeata* began in October, 1914, with the isolation of fifty individuals from a pipetteful of debris taken from a mat of Oedogonium growing on a lotus stem in the Homewood pond, Baltimore. About twenty of these lines died out during the first month, while I was learning the best culture methods. At the end of this time the thirty remaining lines all seemed to be distinct from each other. To keep the cultures within manageable bounds, only the four

largest clones were continued for two months more. In considering inheritance within a population, then, the population consists only of these four clones, numbers 9, 30, 41 and 43, containing in all 1049 individuals.

It was evident, from the very first, that at least two distinct strains existed in my material. A glance at table 1 or figure 4 shows clearly that line 30 differs most decidedly from the other three lines. Their small size, rapid fission rate and high spine number differentiates individuals of this line from the others at first sight.

The other three lines resemble each other more closely. The differences between them are more minute. Line 43 is the most clearly distinct of the three. Its higher fission rate alone would suffice to separate it from the others, and its distinctness is further shown by the slightly smaller size and lower spine number of its members.

TABLE 1

Characters of the diverse lines.

Character	Line 9	Line 30	Line 41	Line 43
No. of individuals	93	749	126	81
Av. length	197.17	149.80	196.32	194.06
Av. breadth	188.25	144.50	188.33	184.02
Av. size index	371.25	216.48	370.19	357.23
Av. form ratio	1.048	1.038	1.044	1.056
Av. mouth size index	45.76	25.75	46.04	40.06
Av. mouth form ratio	.926	.920	.879	.923
Av. spine number	1.161	6.271	1.40	1.062
Av. number of days between two fissions	11.53	6.12	11.31	9.40

The other two lines, 9 and 41, are closely allied. The most striking difference between them lies in the higher average spine number of line 41, shown in table 1 and figure 5. Shell size and form are almost identical, as is also fission rate. The size and form of the mouth show slight differences. To anticipate the conclusions of this paper, it seems not improbable that lines 9 and 41 have been derived ultimately from a common ancestor.

It is to be noted, in connection with table 1, that the characters of size, spine number and fission rate show large differences between diverse clones, while the differences in form of shell and mouth are small.

When one recalls that the original ancestors of these lines were iso-

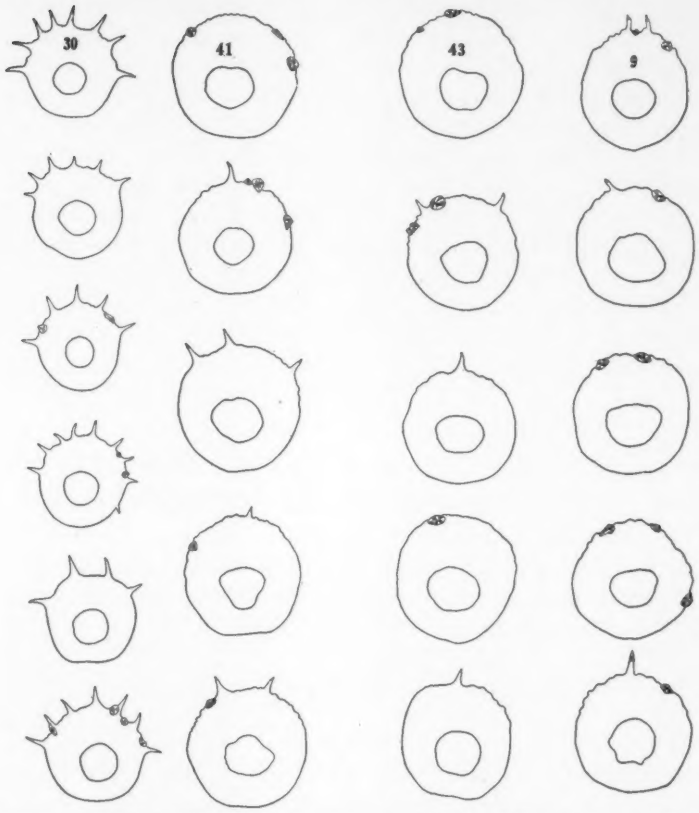


FIGURE 4.—Sample individuals (after camera outlines) of lines 9, 30, 41 and 43.

lated at the same time and from the same place, and that the individuals of the different clones were cultivated at the same time, under the same conditions, the slides containing them intermingled in the same moist chambers, it seems unnecessary to adduce any further evidence that diverse strains, differing in all the characters studied, do exist in Centropyxis.

Ancestral correlations within a population

In order to have a basis for comparison for the coefficients of correlation showing the inheritance of diversities within the single clones, coefficients of correlation were computed to show the degree of such

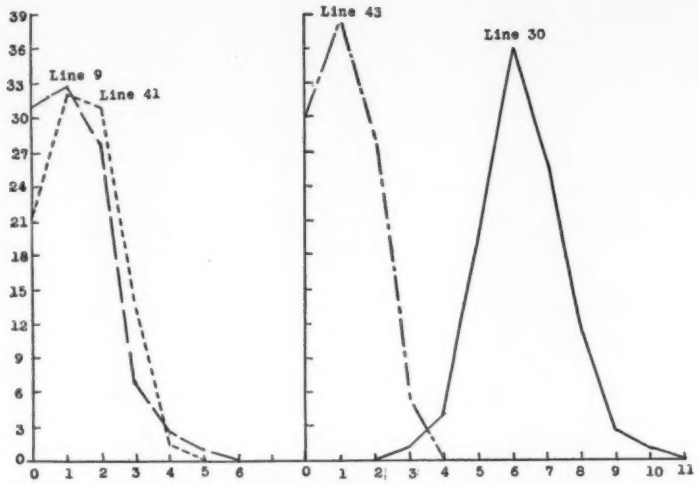


FIGURE 5.—Graphs of the percentage distribution of spine number in lines 9, 30, 41 and 43.

inheritance within the population. In table 2 are given the parental and grandparental coefficients of correlation for all the characters studied. In this table two points stand out clearly:

TABLE 2

Coefficients of correlation for the population.

Character	Parental correlation (1040 individuals)	Grandparental correlation (997 individuals)
Spine number..	.806 ± .007	.785 ± .008
Shell size	.903 ± .004	.773 ± .008
Shell form	.107 ± .022	.019 ± .021
Mouth size	.732 ± .010	.677 ± .011
Mouth form ..	.142 ± .021	.098 ± .021

First, the correlation is very high for shell size and high for spine number and mouth size, but low for the two form characters. This agrees with the conditions found in table 1. There the distinctions between the clones were definite in size and spine characters, but indefinite in form characters. So already two of the characters chosen for study have been practically eliminated for future work. It is perhaps signifi-

cant that these characters are ratios, a kind of character with which much biometrical work has been concerned.

In the second place, it is noteworthy that, in every case, the grandparental coefficient is decidedly smaller than the parental. As PEARSON (1910) has pointed out, according to the pure line hypothesis the offspring should resemble any other individual of the same clone, for example, their grandparent, just as much as they do their parent. PEARSON's main argument against the evidence of the earlier pure line workers is this phenomenon of the diminishing ancestral correlation, which he considers satisfactory evidence that inheritance of variations within the pure line is occurring. It must be noted, however, that the objection to relying solely on the coefficient of correlation for proof of inheritance, because of the effect of a steadily changing environment (see page 177), applies with equal force to this phenomenon.

INHERITANCE WITHIN SINGLE CLONES

Measured by the coefficient of correlation

Ancestral correlations in line 30

Because of its rapid rate of division, many more individuals of line 30 were obtained than of any of the other races. The fact that this single clone contains 749 individuals makes it much the most suitable for statistical study. The other clones, lines 9, 41 and 43, with 93, 126 and 81 individuals, respectively, will be useful only for comparison. The numbers concerned are too small to admit of definite conclusions from these three lines.

In table 3 are given the coefficients of correlation for the first (parental), second (grandparental), third and fourth ancestral generations of line 30. It is at once evident that the five characters considered fall into two groups.

TABLE 3

Coefficients of correlation for line 30.

Character	Ancestral generations			
	1 (755 individuals)	2 (754 individuals)	3 (744 individuals)	4 (693 individuals)
Spine number	.082 ± .024	.034 ± .024	.009 ±. 025	.007 ± .026
Shell size	.253 ± .023	.026 ± .025	.012 ± .025	.019 ± .026
Shell form	.062 ± .025	—.045 ± .025	—.056 ±.025	—.011 ± .026
Mouth size	.024 ± .024	.013 ± .025	—.033 ± .026	—.056 ± .026
Mouth form	.066 ± .024	—.043 ± .025	.066 ± .026	—.008 ± .026

The parental coefficients for spine number and shell size are significant, in comparison with their probable errors. All the coefficients for these two characters are positive, and there is a regular decrease in their size as one passes from more recent to more remote ancestral generations. This evidence points toward the inheritance of variations in size and spine number, even within the single clone.

The other three characters, shell form, mouth size and mouth form, show no such evidence of inheritance. The parental coefficients are less than three times their probable errors, many of the other coefficients are negative, and no diminishing ancestral correlation is to be found. It can be said only that while variations in these characters may possibly be inherited, this table gives no evidence leading to such a conclusion.

Ancestral correlations in the other clones

In table 4 are given, for comparison, the coefficients of correlation for the first and second ancestral generations in lines 9, 41 and 43.

In all of these lines, the numbers available were so small that no definite conclusions one way or the other can be drawn from them. As a glance at the table will show, they give no evidence for the inheritance of variations within the clone. Many of the coefficients, in both generations, are negative, hardly any are large enough to be significant, and in the few cases where there is a diminishing ancestral correlation, it is probably due to chance alone. One thing, only, is worth noting. The coefficients for spine number, in both generations, are all positive. This fact has no significance by itself, but, in comparison with the conditions shown by all the other characters, it may be considered to support the conclusion that variations in spine number may be inherited within the clone.

To sum up the purely biometrical part of the work, we may say:

1. Diverse clones exist in Centropyxis.

2. Within a population, diversities in size of shell and of mouth, and in spine number, are decidedly inherited, while diversities in form of shell and of mouth are only slightly inherited.

3. Within single clones, the evidence from coefficients of correlation indicates that variations in shell size and in spine number are inherited. The evidence that variations in the other characters are inherited is weak or lacking.

It was now decided to choose a single character for intensive study of the effect of selection. The statistical work has eliminated the two form characters, as showing only slight correlation even in a population.

There remain only spine number and the two size characters, which must be considered together, since they are closely correlated.

TABLE 4

Coefficients of correlation for lines 9, 41 and 43.

Line 9

Character	Parental correlation (93 individuals)	Grandparental correlation (81 individuals)
Spine number .	.148 ±.069	.041 ±.075
Shell size	—.121 ±.069	—.060 ±.075
Shell form	—.135 ±.069	.120 ±.074
Mouth size ...	—.071 ±.069	—.155 ±.073
Mouth form ..	—.053 ±.070	—.252 ±.070

Line 41

Character	Parental correlation (130 individuals)	Grandparental correlation (116 individuals)
Spine number .	.006 ±.060	.125 ±.060
Shell size	—.062 ±.060	—.074 ±.063
Shell form	—.057 ±.060	.152 ±.062
Mouth size ...	—.017 ±.060	.117 ±.059
Mouth form ..	.185 ±.058	—.061 ±.060

Line 43

Character	Parental correlation (81 individuals)	Grandparental correlation (69 individuals)
Spine number .	.144 ±.073	.215 ±.076
Shell size	.088 ±.075	—.019 ±.080
Shell form	—.082 ±.075	—.053 ±.080
Mouth size ...	—.148 ±.074	.117 ±.079
Mouth form ..	—.080 ±.075	.240 ±.075

Shell size, as has been shown, gives the highest parental coefficient of correlation, both in the population and in line 30. In the other lines

the coefficient was often negative. Spine number shows a decided correlation in the population and a significant one in line 30, and in all the other lines the coefficient was always positive. It is easy to imagine a way in which shell size could be "inherited" in a purely mechanical sense, since the old shell acts as a limit to the possible growth of the organism within it, and this organism then serves as a mold over the surface of which the new shell is secreted. Finally, spine number is the character which appears to have the least relation to the physiological activity of the organism, and is the one which can be most accurately and easily determined. For these reasons, spine number was chosen as the subject for selection experiments.

After the objections which have just been raised to the use of shell size as a subject for selection experiments, it is advisable to determine whether variations in shell size are correlated with variations in spine number. This is especially necessary, since in our population we find that the large lines have few spines and the small lines many spines. Within the clone, however, this does not hold. The coefficient of correlation between shell size and spine number for the 745 individuals of line 30 is $.019 \pm .025$, that is, for all practical purposes, zero.

Inheritance proved to occur by selection experiments

Selection experiments 1 and 2; contradictory results of two brief experiments in mass selection

In order to bring out clearly the reasons which led me to abandon mass selection for individual selection based on a progeny test, it seems worth while to give a brief account of two experiments in mass selection which came to an abrupt and unexpected end. The first of these experiments was ended by an epidemic of sexual reproduction, the second by a period of extremely hot weather which probably acted indirectly by accelerating the multiplication of undesirable bacteria.

Neither of these experiments lasted long enough for the application of the real test of their effectiveness, the free multiplication of two altered strains in comparison with each other. But since selection consists only in allowing certain individuals to breed and denying that privilege to others, a good idea of whether selection is being effective can be gained by comparing the average spine number of the progeny produced by high- and low-selected groups during the same period. Such comparisons for selection experiments 1 and 2 are given in tables 5 and 6.

Selection experiment 1. Table 5 shows the progressive effect of selec-

tion in a small many-spined clone closely resembling line 30. The progenitor of this race was isolated from algae taken from the Homewood pond on October 8, 1914. On November 9, 52 individuals had been secured by the multiplication of the original one, and selection began. On December 14 an epidemic of sexual reproduction put an end to the experiment.

TABLE 5

Selection experiment 1, mass selection.
High series: Parents with 6 or more spines.
Low series: Parents with 5 or less spines.
Mean of clone (52 individuals) when selection began: 6.56 spines.

Between selections	Progeny produced in				Difference between high and low means
	High series		Low series		
	Individuals	Mean spine number	Individuals	Mean spine number	
1 and 2.....	49	6.57 ±.10	47	6.35 ±.10	.22 ±.14
2 and 3.....	30	7.36 ±.12	23	5.75 ±.14	1.61 ±.18
3 and 4.....	9	7.76 ±.22	7	5.50 ±.25	2.26 ±.32

In this experiment simple mass selection was used. The clone was divided into two parts, the individuals with six or more spines being classed as high, those with five or less as low. In each group only those progeny whose spine numbers lay on the same side of the mean (6.56 spines) as their parent's were allowed to reproduce.

As table 5 shows, the difference in mean spine number between the progeny produced by the high and low groups was only .22 of a spine after the first selection, but rose to 1.61 spines after the second, and to 2.26 spines after the third. Apparently, then, in this clone, three selections sufficed to isolate within the clone high and low strains with a mean spine number of about 7.75 and 5.5 spines, respectively. This quick and decided effect of selection was most unexpected, and it is most unfortunate that the onset of sexual reproduction ended this experiment so prematurely. The numbers involved are too small to allow of any definite conclusion from an experiment of such short duration.

Selection experiment 2. In table 6 is shown the course of a similar experiment in a clone with fewer spines and of larger size. The progenitor of this clone was isolated on July 3, 1915, from among algae at-

tached to a dead stick in an inlet of Nobska pond near Woods Hole, Mass. Selection was started on July 28, when the clone contained 55 individuals. The last individual of this race died on August 16, after a period of very hot weather.

TABLE 6

Selection experiment 2; mass selection.
High series: Parents with 2 or more spines.
Low series: Parents with 1 or 0 spines.
Mean of clone (55 individuals) when selection began: 1.51 spines.

Between selections	Progeny produced in				Difference between high and low means
	High series		Low series		
	Individuals	Mean spine number	Individuals	Mean spine number	
1 and 2	25	1.96 ±.14	29	0.31 ±.13	1.65 ±.19
2 and 3	5	1.40 ±.30	9	0.00	1.40 ±.30
3 and 4			5	0.40 ±.30	

When selection began, the mean spine number of the clone was 1.51 spines. The high and low groups therefore consisted of individuals with two or more and one or no spines, respectively. The method of selection was the same as in experiment 1. Although the clone died out before more than one or two selections could be made in most of the lines, table 6 shows that this clone behaved very differently from the one used in experiment 1. The first selection gave a decided difference (1.65 spines) between high and low groups, as against a very slight one in the first experiment (.22 spines). And after the first selection no further result was noticeable. This differs decidedly from the steady progressive effect which was found in experiment 1.

In both these experiments mass selection was used. In fact, in almost all the experiments on the effect of selection within pure lines or clones, mass selection has been used exclusively. Mass selection is based entirely on the external appearance of the individual, and it is evident that the degree of effectiveness of the selection depends very largely on the extent to which the genetic constitution of the individual resembles its external aspect. Formerly mass selection within populations was extensively practiced, and one of the greatest achievements of the pure line workers has been the pointing out that by selecting on a basis of the character of the progeny produced by an individual, the same results

can be obtained with much fewer selections. This is true, of course, because the average character of the progeny of an individual gives a much more accurate test of its hereditary constitution than does its external appearance.

If this is true in a population, may it not also be true within a clone? And if one seeks for an effect of selection within a clone, why not use the type of selection which has been found to be most effective in populations?

Selection experiment 3; quick effect of individual selection based on progeny

In accordance with the train of reasoning set forth above, individual selection based on the average spine number of the progeny was used in my next experiment. On October 10, 1915, an individual was isolated from the Homewood pond. After it had produced five progeny it was discarded. Each of these progeny, in turn, produced five offspring and was then discarded. Of these five sets of offspring, the set with the highest average spine number and the set with the lowest were allowed to produce five progeny each. The other three sets were discarded. Then another selection was made, the set of progeny with the highest average spine number being retained on the high side, and *vice versa* on the low side. This method of selection was continued for another generation.

TABLE 7

Selection experiment 3: Individual selection, based on the character of the progeny.

Generation 1:	5 (1.0)
Generation 2:	5a (0.8) 5b (0.3) 5c (0.2)
Generation 3:	
High series	5a1 (1.5) 5a3 (0.0) 5a4 (1.0) 5a5 (0.0)
Low series	5c1 (0.3) 5c2 (0.2) 5c5 (1.0)
Generation 4:	
High series	5a1a (1.2) 5a1c (0.0) 5a1d (1.0)
Low series	5c2a (0.2) 5c2b (1.0) 5c2c (0.6) 5c2d (0.3)
Generation 5:	
High series	5a1a1 (1.8) 5a1a2 (1.7) 5a1a4 (1.0) 5a1a5 (1.2)
Low series	5c2a2 (0.2) 5s2a3 (0.4) 5s2a5 (0.6)
Generation 6 and following generations:	
High series—56 individuals derived from 5a1a1—average spine number	1.68±.09
Low series—51 individuals derived from 5c2a2—average spine number	0.22±.002
Difference between high and low means	1.46±.085

Table 7 shows the pedigree of this experiment. In this table, 5a, 5b and 5c are the progeny of individual 5; 5a1, 5a3, 5a4 and 5a5 are the

progeny of 5a; and so on. The numbers in parentheses, following the numbers which represent the individuals, are the average spine numbers of their five (in a few cases, six) progeny. All individuals which did not produce at least five progeny have been omitted from the table.

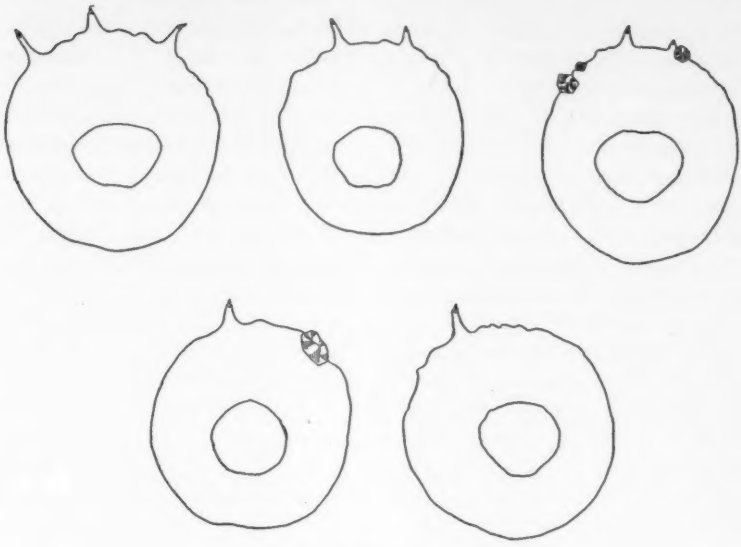
On December 14, after four selections had been made in both high and low groups, selection was discontinued, and the final individuals 5a1a1 and 5c2a2 and their progeny were allowed to multiply freely. Because of cold weather, the fission rate was very low, and when about fifty individuals had been produced in each group the experiment was ended on February 3, 1916.

The numbers concerned in this experiment are not large, but the difference between the means of the two final groups (1.46 spines) is so great in proportion to its probable error (.085) that there can be no doubt that in this case selection has isolated, within a single clone, two strains with decidedly different average spine numbers. In figure 6 are shown a few characteristic members of the two groups, from camera outlines.

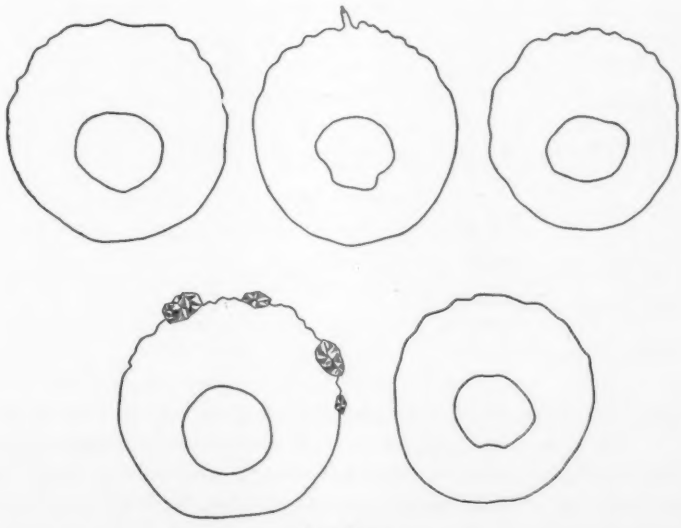
In this experiment there is one possibility of error. When one is selecting individuals instead of masses of individuals,—a single line instead of a number of lines,—an excellent opportunity is offered for the multiplication of any mutation that may occur. Recognizing this fact, and being now quite convinced in my own mind that selection within the clone was having an effect, I turned to mass selection once more. In mass selection, with many parallel lines, there is no chance for the extreme multiplication of a mutation, and if one does occur it changes the final result only to a very slight degree. And if selection within a clone is really effective, mass selection on a large scale, in a favorable race, should show such an effect. (By a favorable race, I mean, of course, one in which the external appearance of an individual is a fairly accurate index of its genetic constitution.)

Selection experiment 4; decided effect of mass selection in a favorable race

On June 29, 1916, an individual of Centropyxis was isolated from material collected from a freshwater pond on Cuttyhunk Island near Woods Hole, Mass., and was allowed to multiply. The course of this experiment is summarized in table 8. On August 1, when the line comprised 133 individuals, it was divided into two groups. This race was of large size, with a medium number of spines. On August 1 the average spine number was almost exactly 2.5 spines. All individuals with 0, 1



High Group.



Low Group.

FIGURE 6.—Sample individuals of high and low groups at the end of selection experiment 3.

or 2 spines, 63 in number, were set aside as progenitors of low lines, and all individuals with 3 or 4 spines (there were 70 of these) became progenitors of high lines. As soon as any of these individuals divided, the new one was examined. In the low lines, if the new individual had 3 or 4 spines, it was destroyed; but if it had 0, 1 or 2 spines, it was retained and its parent destroyed. In the high lines, of course, the reverse procedure was followed. Such selection was continued until August 26, only a few days before I had to leave Woods Hole. From August 26 to September 3, inclusive, no selection was practiced, and all the remaining individuals of both groups were allowed to multiply freely.

TABLE 8

Selection experiment 4; mass selection.
High series: Parents with 3 or 4 spines.
Low series: Parents with 0, 1 or 2 spines.

Period of	Time	Number of individuals	Average spine number	Difference between high and low lines
Multiplication	June 29-July 8	9	3.11 ±.22	
	July 9-18	28	2.39 ±.14	
	July 19-30	96	2.44 ±.08	
Selection	Aug. 1-10			
	High lines	146	2.71 ±.05	.55 ±.09
	Low lines	133	2.16 ±.07	
	Aug. 11-20			
	High lines	167	2.55 ±.05	.48 ±.09
	Low lines	146	2.07 ±.07	
	Aug. 21-25			
	High lines	89	2.77 ±.06	1.00 ±.09
	Low lines	79	1.77 ±.07	
Multiplication after selection	Aug. 26-Sept. 3			
	High lines	192	2.75 ±.05	.99 ±.069
	Low lines	180	1.76 ±.05	

During this time more than 175 offspring were produced by each group. The average spine numbers of these two sets of progeny differ by nearly a whole spine, with a probable error so small as to be negligible. This experiment, I think, proves conclusively that the effect of selection which has been demonstrated in experiment 3 is due to the inheritance of minute variations in spine number within the clone.

In this mass selection experiment, the appearance of a mutation has little effect on the ultimate result. Something comparable to a mutation did, in fact, appear in the low series of experiment 4. During the first period of multiplication, individual 1d1, with one spine, began producing progeny all of which had no spines. Progeny with no spines were comparatively rare among the other low lines. All these progeny of 1d1 also produced progeny with no spines, predominantly, but with a few one-spined progeny. The early appearance of this mutation gave it greater prominence than it would have enjoyed later on, since 1d1 and three of its offspring were included among the progenitors of low lines. In all, 39 individuals can be traced back to 1d1 as their original ancestor. Of these, 33 had no spines and 6 had one spine. But the effect on the final result of the experiment is inconsiderable. If all of the offspring of the mutation are left out of the table, the number of individuals produced by the low lines between August 26 and September 3 is reduced to 168, with an average spine number of 1.867. This still gives a difference between the average spine numbers of the high and low groups of $.88 \pm .07$.

The very favorable character of the race employed in experiment 4, that is, the close correspondence between its external appearance and genetic constitution, is shown by the high correlation between parent and progeny. The coefficient of correlation between parents and progeny, for spine number, is $.376 \pm .017$ for the 1277 individuals of this race. This is the highest coefficient of correlation within a clone that has been obtained in the course of my experiments. If the mutation 1d1 and its offspring are omitted, the coefficient becomes $.282 \pm .018$.

CONCLUSIONS

Results of the present investigation

The present study of inheritance in Centropyxis may be divided into two parts, the first concerned with a statistical analysis of inheritance within the population and within single clones, the second with an attempt to test the inheritance of variations in a single definite character within the clone, by the method of selection.

The results of the biometrical study of inheritance in Centropyxis may be summarized as follows:

1. There exist in the species, *Centropyxis aculeata,* many diverse strains or clones which, under identical environmental conditions, differ from each other decidedly in spine number, shell size, mouth size and fission rate, but which differ only slightly in shell form and mouth form.

2. In a population, the coefficients of correlation show that variations in spine number, shell size and mouth size are strongly inherited, while variations in shell form and mouth form are weakly inherited.

3. In a single clone, the coefficients of correlation show that variations in spine number and shell size are probably inherited.

4. If a diminishing ancestral correlation is considered a criterion of inheritance of variations within a clone, the coefficients of correlation of the population give such evidence of inheritance for all the characters studied, and the coefficients of correlation within a clone give such evidence for spine number and shell size.

5. From the evidence adduced under 2, 3 and 4 above, it was concluded that variations in spine number and shell size are inherited within the clone. Since shell size might be "inherited" without the mediation of the nucleus, spine number was considered the most favorable character for use in selection experiments.

The results of the experiments in the selection of variations in spine number may be summarized as follows.

6. In the brief experiments 1 and 2, mass selection based on external appearance gave contradictory results in two different races. In explanation of this, it is assumed that in different races the degree of correlation between the external appearance of the individual and its genetic constitution is diverse; in some the two are highly correlated, in others but little.

7. In experiment 3, individual selection based on the average spine number of the progeny of the individual under consideration resulted in a rapid isolation of two decidedly different strains within a single clone.

8. Since the result in experiment 3 might have been due to the appearance and preservation of a mutation, mass selection was again tried. In experiment 4, on a very favorable race (as is shown by its high parental correlation), mass selection based on the external appearance of the individual was sufficiently effective to isolate two distinct strains from within a single clone. In this experiment 1277 individuals were obtained, all coming originally from a single specimen.

9. In this experiment 4, something resembling a mutation did appear. Its 39 progeny were traced out, and it was shown that their elimination from the record makes no essential change in the final result.

The present status of the "pure line" hypothesis

In considering the bearing of this work and other similar investigations on the concept of the "pure line," one must carefully distinguish

between the two parts of which this hypothesis is composed. The term "pure line" was originally applied to groups of individuals derived by self-fertilization from a common homozygous ancestor. Such lines within a single species prove to be relatively constant diverse strains. That such relatively constant strains do occur in a great variety of plant and animal species, is an observed fact, and has been confirmed by every investigator who has entered this field. The further idea, that within one of these "pure lines" no variation in genetic constitution is possible, except by a sudden mutation, large or small, is not direct observation but hypothesis. It is an hypothesis, to be sure, which was based on all the experimental evidence available at the time it was formulated, but it is still an hypothesis, subject to revision at any time when new experimental results demand it.

For some years, the great majority of the experimental results tended to confirm this hypothesis, and it gained such a firm footing that all the contradictory cases were dismissed as aberrant types, results of a failure to properly control the environment, observational errors, etc. But of late the tide seems to be turning somewhat. STOUT (1915) in Coleus, STOCKING (1915) in Paramecium, MIDDLETON (1915) in Stylonychia, and JENNINGS (1916) in Difflugia, have all shown that minute heritable variations do occur within clones derived from a single ancestor by vegetative multiplication. In the present work on Centropyxis, the same conclusion is reached.

We must admit, it seems to me, that in these plants and protozoans reproducing asexually, small variations in genetic constitution do continually occur. How does this affect the "pure line" hypothesis? The issue can be met in two ways.

In the first place, it is still possible to reaffirm the entire validity of the original pure line hypothesis. The new conflicting results, like previous ones, can still be explained away as due to complicating factors. For example, the inheritance of abnormalities in Paramecium (STOCKING 1915) can be considered to follow from an abnormal condition of the macro-nucleus; the inheritance of variations in fission rate in Stylonychia (MIDDLETON 1915) might be due to the accumulation of waste products in the cytoplasm of the slowly-dividing, large-sized group. And in Difflugia (JENNINGS 1916) and Centropyxis (the present paper), until we know more about the process of cell division in these organisms, the observed inheritance of variations can be explained by the assumption that a process of somatic segregation or fractionation of the chromidial body takes place at each cell division, as has been suggested by MORGAN

(1916, p. 185). Other similar cases could doubtless be supplied with other explanations along the same lines.

But there is another viewpoint, alternative to the first one, which seems to me to be preferable. According to this idea, one may assert that the pure line concept is correct on the average, only; that it is a mathematically correct expression of the mean result of inheritance under natural conditions. But, just as the Galton-Pearson law of ancestral inheritance, though accurate mathematically, does not fit the details of Mendelian heredity, on the average result of which it is presumably based, so the pure line hypothesis, though true for average results, may be thought of as not holding in individual cases. Or, to look at the question from another side, just as species were found to consist of a number of pure lines, diverse from each other in minor details of genetic constitution, so we may think of pure lines as made up of a number of individuals, differing from each other in even smaller details which are, nevertheless, hereditary.

According to this idea, we can assume that each individual inherits, in general outlines, the genetic constitution of its species; in smaller details, that of its pure line; in still more minute details, that of its individual parent; and yet may differ from this parent in some hereditary points. There is a growing inclination among geneticists to designate all such hereditary differences, mutations, regardless of their magnitude, but I am opposed to this usage and would limit the word mutation to hereditary differences which are fairly large. If such differences are very minute, they can be termed genetic fluctuations, micro-mutations, or any other term which is found to meet the needs of those who work in this field.

So far as our present knowledge goes, either of these two views may be championed with equal success. The final decision between them must be left to the experimental results of the future.

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APPENDIX

Some important correlation tables

TABLE 9

Correlation between spine numbers of parents and their progeny in the population.

Parents \ Progeny	0	1	2	3	4	5	6	7	8	9	10	Totals
0	25	33	18	2	2							80
1	26	37	30	6	3							102
2	19	41	19	4	4	1						88
3	6	12	6	3	.1	2	2	1				33
4		3		2		6	15	7	1			34
5		1		5	10	33	43	33	17	3	3	148
6				8	8	51	109	49	31	13	2	271
7				8	2	28	85	41	23	6	2	195
8				3	1	18	32	15	9	2	1	81
9					1		8	8	2	1		20
10							1	1	2			4
Totals	76	127	73	41	32	139	295	155	85	25	8	1056

TABLE 10

Correlation between shell size indices of parents and their progeny in the population.

Parents \ Progeny	17	19	21	23	25	27	29	31	33	35	37	39	41	43	45	Totals
17	2	5	16	5												28
19	8	33	71	16	4	1	4									137
21	6	69	192	71	26	1	7									372
23		20	85	48	15	6	3									177
25			15	6	3	2	1		1							28
27						1	1		1							3
29						1					1	1	2			5
31									7	5	5	8	1		1	27
33							3	2	8	14	7	13	2		4	53
35								3	12	10	12	21	2	1	3	64
37							6	2	12	15	7	23	3		2	70
39							1		6	8	7	20	3		3	48
41							1		1	3	6	5		1		17
43										1	2	4				7
45										1		2				3
Totals	16	127	379	146	48	12	27	7	48	57	47	97	13	2	13	1039

TABLE 11

Correlation between shell size indices of grandparents and progeny in the population.

Grandparents \ Progeny	17	19	21	23	25	27	29	31	33	35	37	39	41	43	45	Totals
17		4	13	6	1	1	2									27
19	1	22	71	15	4	14	10									137
21	1	49	201	59	28	23	14									375
23		21	96	30	11	12	9									179
25		1	17	1	1	3	3									26
27							1									1
29							2				1			1		4
31							1	1	5		4	7			4	22
33							2		6	7	9	19			6	49
35							3		5	9	11	21		1	7	57
37							3	5	8	7	8	24			6	61
39							1		2	6	5	19		1	5	39
41							2		1	3	4	5			2	17
43							1	1	1			2			1	6
45								1								1
Totals	2	97	398	111	45	53	54	8	28	32	42	97	0	3	31	1001

TABLE 12

Correlation between spine numbers of parents and progeny in line 30.

Parents \ Progeny	3	4	5	6	7	8	9	10	Totals
3	1		2	2	1				6
4	2		6	15	7	1			31
5	5	10	33	43	33	17	3	3	147
6	8	8	51	109	49	31	13	2	271
7	8	2	28	85	41	23	6	2	195
8	3	1	18	32	15	9	2	1	81
9		1		8	8	2	1		20
10				1	1	2			4
Totals	27	22	138	295	155	85	25	8	755

TABLE 13

Correlation between spine numbers of grandparents and progeny in line 30.

Grandparents \ Progeny	3	4	5	6	7	8	9	Totals
3	1	1	1	2				5
4	6	3	6	6	6	4		31
5	7	5	21	70	33	7	3	146
6	22	11	45	119	55	10	9	271
7	16	9	39	81	37	10	4	196
8	6	3	14	36	14	6	2	81
9		1	3	10	4	1	1	20
10				3	1			4
Totals	58	33	129	327	150	38	19	754

TABLE 14

Correlation between the spine numbers of great-grandparents and progeny in line 30.

Great-grandparents \ Progeny	3	4	5	6	7	8	9	Totals
3	3	1	1					5
4	5	1	5	14	3	1	1	30
5	26	10	27	60	20	2		145
6	39	16	52	128	26	5	2	268
7	37	11	33	85	21	3	2	192
8	14	2	17	37	6	2	2	80
9	7		2	9	2			20
10				4				4
Totals	131	41	137	337	78	13	7	744

TABLE 15

Correlation between shell-size indices of parents and progeny in line 30.

Parents \ Progeny	16	17	18	19	20	21	22	23	24	25	26	27	28	29	Totals
16								1							1
17				1		3	1	1							6
18		2		2	2	3	9	1	2						21
19		2	1	6	9	11	8	2		2					41
20		3	2	8	10	22	30	10	4	2		1		4	96
21		2	1	11	25	44	53	21	9	12				3	181
22		2	1	9	24	41	53	31	10	14		1		4	190
23				2	11	21	38	23	10	7	3	4	1	3	123
24				1	6	10	16	13	2	5		1			54
25						4	8	3	1	2		2		1	21
26						1	2	1	1	1					6
27														1	1
28												1			1
29												1			1
Totals	11	5	40	87	160	218	107	39	45	2	11	1	16		743

TABLE 16

Correlation between spine numbers of parents and progeny in line 9.

Parents	Progeny 0	1	2	3	4	Totals
0	10	11	6		1	28
1	7	8	12	2	1	30
2	5	11	8	1	1	26
3	1	2	2		1	6
4		2				2
Totals	23	34	28	3	4	92

TABLE 17

Correlation between spine numbers of parents and progeny in line 41.

Parents	Progeny 0	1	2	3	4	5	Totals
0	9	8	8	2	1		28
1	16	14	8	2	2		42
2	12	15	7	2	3	1	40
3	5	7	3	2			17
4		1					1
5		1					1
Totals	42	46	26	8	6	1	129

TABLE 18

Correlation between spine numbers of parents and progeny in line 43.

Parents	Progeny 0	1	2	3	Totals
0	6	14	4		24
1	3	15	10	2	30
2	2	15	4	1	22
3		3	1		4
Totals	11	47	19	3	80

TABLE 19

Correlation between spine numbers of parents and progeny during selection experiment 4.

Parents	Progeny 0	1	2	3	4	Totals
0	26	14				40
1	11	116	82	78	24	311
2		70	70	106	33	279
3	1	56	82	204	98	441
4		41	34	84	46	205
Totals	38	297	268	472	201	1276